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PROLOGO

EL NACIMIENTO de Acta *Physiologica Latinoamericana* se debe a un esfuerzo coordinado de los fisiólogos de la América Latina para intercambiar los resultados de sus investigaciones científicas entre ellos mismos y con los fisiólogos de los demás países. En esta forma procurarán contribuir —de manera más regular, sistemática y organizada que hasta ahora— al adelanto continuo de las ciencias fisiológicas

Cierto es que durante mucho tiempo la producción científica original de la América latina fué muy escasa y fragmentaria, no siempre importante, y con frecuencia demostró conocimientos insuficientes y espíritu crítico inmaduro. Pero en las últimas décadas se ha producido un despertar y un adelanto manifiestos. Paulatinamente aparecieron hombres cada vez mejor formados y que se dedicaron preferente o exclusivamente a la investigación científica; su número está creciendo cada vez más y en más países; algunos formaron discípulos y aun escuelas. Este adelanto se ha acelerado considerablemente porque muchos fisiólogos de la América latina han perfeccionado sus conocimientos y su capacidad en el extranjero, bajo la dirección de grandes maestros que fueron hombres de ciencia sobresalientes. Por todas estas razones, va siendo cada vez mayor el número de investigadores latinoamericanos, su labor es más importante y más diversificada y la calidad de los trabajos mejora en grado apreciable.

Los motivos que han decidido a iniciar esta publicación, que desde hace años era una necesidad sentida y que no podía postergarse indefinidamente, han sido, principalmente: a) la existencia de trabajos originales de suficiente importancia; b) el aumento constante de esa producción; c) la necesidad de corregir el defectuoso conocimiento de esos trabajos entre los mismos fisiólogos de la América latina; d) su desconocimiento muy grande, por los fisiólogos de otros países, inclusive los de la América del Norte, que difícilmente podían encontrarlos; e) la dificultad creciente para hacerlos ingresar a la literatura mundial circulante y activa, pues las revistas de los grandes países sufren escasez de espacio, aun para los trabajos de su misma nación, y no pueden ni podrán aceptar indefinidamente las contribuciones latinoamericanas.

Una revista de elevada responsabilidad como pretende ser ésta, debe

ajustar sus publicaciones a ciertas reglas que mantengan su alto nivel. Por ello se propone publicar solamente trabajos originales importantes y suficientemente completos, y no los trabajos fragmentarios o parciales que pueden hallar cabida en las revistas de las sociedades donde se presentan y otras publicaciones del país de los autores. También publicará una síntesis ordenada de una serie de publicaciones parciales de un autor o escuela, cuando la investigación haya adquirido suficiente madurez o sea bastante completa. Estas síntesis serán resúmenes de labor original y no revistas mundiales del problema. En algunos casos podrá publicarse una síntesis de una serie de trabajos originales publicados en más de un país de América latina y por varios autores, cuando se trate de un tema nuevo, estudiado exclusiva o preferentemente en países latinoamericanos o sea las naciones de América que hablan en español, portugués o francés.

Mientras persista la sorprendente ignorancia de las lenguas latinas en los países de mayor actividad científica, será conveniente que los trabajos más importantes aparezcan preferentemente en las lenguas de los mismos, o bien en lenguas latinas pero con un resumen en inglés y otro idioma de amplia circulación actual en el mundo de la ciencia.

El logro de estos fines exigirá una tarea cuidadosa de selección a cargo de los comités locales y de crítica previa para que los autores se ajusten a las normas editoriales, sean claros en sus exposiciones y den pruebas suficientes de sus afirmaciones o conclusiones.

Esta revista se propone difundir las adiciones nuevas a los conocimientos actuales, ser un estímulo, educar, favorecer el aumento y la mejora paulatina de la buena producción y la disminución de la que no lo sea. Estas tareas representan una responsabilidad muy seria ante los hombres del presente y los del futuro.

Las verdades científicas tienen valor universal, por encima de las fronteras geográficas o políticas. Pero cada país y grupo humano tiene la obligación moral de contribuir al aumento de los conocimientos científicos en la medida más intensa que le permitan sus fuerzas. La América latina, que ha sobrepasado la infancia y entrado en la mayor edad, no puede eludir ese deber de creación y de propagación de los conocimientos originales que tienden a elevar el espíritu y beneficiar a la humanidad.

BERNARDO A. HOUSSAY.

FOREWORD

THE APPEARANCE of *Acta Physiologica Latinoamericana* is due to the coordinated effort of Latin-American physiologists, in order to satisfy their need of communicating to each other and to physiologists in other countries the result of their scientific work. They will thus endeavour to contribute to the advancement of physiological science in a more regular, systematic and organized way.

For many years the scientific output of Latin America was scanty, fragmentary, of little importance and frequently betrayed insufficient knowledge of the questions discussed and an inmature critical capacity. In the last few decades, however, there has been an awakening and considerable improvement. Gradually men with better training have entered the field of physiological research and have made it their principal, in some cases their full time, occupation. Some of them have trained younger men and a few have created what may be called schools of physiology. Their number is increasing and they are beginning to appear in more Latin American countries. Advancement of physiological knowledge in this part of the world has been favoured by the fact that many Latin American physiologists have completed their training and perfected their knowledge in foreign countries, under the direction of eminent scientists. An increasing amount of more important and varied original work of much higher quality has been the result.

The need of a periodical such as *Acta Physiologica Latinoamericana* has been felt now for several years owing to: a) the increasing output of sufficiently important original work; b) the necessity of making this work known to other physiologists in Latin America; c) the fact it remains almost unknown to physiologists in other countries, including

those of North America, because the publications in which it is reported are not easily available; d) the increasing difficulty of obtaining space in the overcrowded scientific periodicals of other countries, which form part of the actively circulating world literature, and which cannot be expected to give unlimitated hospitality to Latin American contributions when they scarcely have room for work done in their own country.

A scientific periodical which wishes to acquire and maintain a reputation must lay down certain rules which establish a high standard for its publications. *Acta Physiologica Latinoamericana* will accept only reports of important original work, which has been sufficiently completed. It will not accept fragmentary contributions, which may be appropriate for communication to a learned society and can appear in its minutes, or may find acceptance in local periodicals. It will also publish papers which give an orderly review of work reported fragmentarily elsewhere by an author or a group of authors, in order to offer the reader a general idea of research which is being done and has reached a certain degree of maturity. These papers will not be reviews of the world literature on the subject, but reports of original work of the authors. In some cases these papers will summarize work done on the same problem by several authors in different Latin American countries.

As long as the surprising ignorance of Latin languages persists in those countries in which scientific activity is most developed, it will be necessary to publish the more important papers in the language of those countries, or to add a summary in English and in another language widely understood in the scientific world.

Local editorial committees will have to select the papers carefully; submitting them to critical analysis in order to see they conform to the established rules, report the work done clearly and concisely and give adequate proof of the conclusions which have been reached.

Acta Physiologica Latinoamericana by publishing new additions to knowledge, hopes to stimulate research of high quality, and to discourage work of poor quality, which is of little or no value for the advancement of science. It is realized this means undertaking serious responsibility.

Scientific truth has universal value, which transcends geographical and political frontiers; but every country or group of men have the moral obligation of contributing to the increase of scientific knowledge as far as their resources allow. Latin America has left its infancy behind and having reached maturity must fulfill its duty of doing original scientific work which improves the mind and increases the welfare of humanity.

BERNARDO A. HOUSSAY.

THE RENIN CONTENT OF KIDNEY

By J. C. Fasciolo

(Instituto de Fisiología, Universidad Nacional de Tucumán)

I. In search of a culprit

Tigerstedt and Bergman (1), in 1898, showed that saline extracts of kidney raise the arterial pressure when injected intravenously, the pressor substance being named "renin".

Little attention was paid to this discovery until 1934, when Goldblatt, Lynch, Hanzal and Summerville (2), showed that arterial hypertension could be produced in dogs by constriction of the renal artery.

Experimental evidence indicated that the hypertension was produced by a chemical mediator formed and secreted by the kidney. Naturally, renin was the suspect Nº 1. As in murder cases, renin had opportunity, since it is present in kidney tissue; it had also a motive, since the elevation of the arterial pressure may be looked upon as a compensatory mechanism for the renal ischemia. Finally, renin raises arterial blood pressure by a mechanism similar to the one which produces hypertension in the ischemic animal. In other words, it seemed that the culprit could also be recognised by the way it worked.

The evidences of renin being the cause of renal hypertension are the following. Here we shall consider only experimental renal hypertension, since the mechanism of human hypertension may be different.

a) Acute renal ischemia, complete or partial, produces liberation of renin into the blood (3, 4).

b) In shock, which causes kidney ischemia, renin was shown to appear in the blood stream (5, 6, 7, 8, 9).

c) Renin has been detected in the blood of dogs for a few days after the kidney has been rendered ischemic, while the blood pressure was rising (10). Haynes and Dexter (11), were able to find renin in the blood of dogs during 2 or 3 weeks after renal ischemia. But later, notwithstanding the further rise of arterial pressure, renin disappears from the circulation. There are some controversial results, notably those of Gollan, Richardson and Goldblatt (12).

Fasciolo and Taquini (13), in 1947, devised a very sensitive method for the estimation of renin, which made possible the recognition of minute amounts in the venous blood of normal persons and dogs, but no increase could be found in chronic hypertension (14). Using this method Braun-Menéndez, Covián and Rapela (15), showed that renin was present in the blood of normal rats, but again no increase was found in perinephritic

hypertensive rats. Summarizing, these facts appear to indicate that the renin content of the blood is increased in the initial stages of experimental renal hypertension, but not in cases of long standing hypertension.

Must we then absolve renin in the light of such evidences?

It is known that renin has an accomplice. In fact, renin has *"per se"* no pressor or vasoconstrictor action. Its vascular action is mediated through the formation of a new substance, ***hypertensin*** (angiotonin) which is produced by the action of renin on a plasma protein, *hypertensi-sinogen* (renin-substrate) ([16, 17]).

Let us analize some possibilities of renin being involved in the mechanism of chronic renal hypertension, without its concentration being increased in the blood:

a) Renin could act upon hypertensinogen, within the kidney and the hypertensin formed pass into the blood and cause an elevation of arterial pressure. Of course, this being the case, the concentration of hypertensin in the blood should increase. Unfortunately the methods available at present preclude the possibility of studying this point *. Some facts speak against the possibility of chronic hypertension being produced by the increased concentration of hypertensin in blood. For instance, extirpation of the ischemic kidney in hypertensive dogs produces a fall in the arterial pressure, but it takes hours to reach the normal levels ([18, 19]). Since the pressor action of hypertensin is of short duration, 3-4 minutes, a much more rapid fall of arterial pressure should be expected if the hypertensin content of the blood were the cause of hypertension.

Furthermore, the graft of ischemic kidneys from hypertensive dogs into the neck of nephrectomized dogs ([20]), produces an elevation of the recipient's blood pressure which persists many minutes after the removal of the grafted kidney (more than 2 hours in some cases). No doubt such rises cannot be ascribed to hypertensin. However, in these experiments the grafted kidney came from dogs with acute or sub-acute hypertension in which, as said before, the renin content of the blood was found to be increased. As a matter of fact, in some cases the rise in blood pressure following the renal graft lasts too long to be attributed to renin **.

b) Renin could be adsorbed in the walls of arterioles and produce its action by the formation of small amounts of hypertensin *"in situ"*. This possibility has received support from the recent work of Introzzi *et al.*([22]), who found renin in heart and liver tissues of shocked dogs***.

c) The hypertensive animal could be more sensitive to renin, as is the case of bilaterally nephrectomized animals, but in this event the participation of renin will be indirect.

No doubt a better knowledge of the changes of renin within the kidney under different conditions will help to clarify the problem. For

(*) We have been unable to detect hypertensin in the blood of a dog after the injection of a unit of hypertensin, although the blood was drawn at the height of the pressor response.

(**) Shipley and Helmer ([21]) believe that the kidney produces a substance capable of producing a pressor response much more sustained than that due to renin.

(***) We were however unable to confirm these findings. (Suárez, J. R., Fasciolo, J. C., unpublished data).

instance, in the case of hypothesis a, (increased secretion of hypertensin by the ischemic kidney) the activity of renin within the kidney should be enhanced by renal ischemia, to increase the secretion of hypertensin.

Renin in the Kidney

Methods for the determination of renin. — Three general principles have been employed for the estimation of renin:

1) *Direct method.* — Renin is extracted from the kidney, partially purified and its pressor action assayed by intravenous injection. This method has several shortcomings: 1) It lacks specificity; 2) Crude kidney extracts like other tissue extracts have a strong depressor action which may mask the pressor effects; 3) Renin produces tachyphylaxis, which makes difficult the comparison of the pressor response.

2) *Method based on the formation of hypertensin* (23). — The hypertensin formed by incubating small amounts of renin with an excess of hypertensinogen is proportional to the concentration of renin. Hypertensin can be estimated by its pressor or vaso constrictor action. This method is very sensitive and specific. It has the disadvantage that hypertensinase must be destroyed before incubation, procedure which may produce some inactivation of renin.

3) *Method based on the destruction of hypertensinogen* (24). — In this procedure the amount of hypertensinogen which disappears as a result of the action of renin is measured. It has the advantage of not requiring the destruction of hypertensinase.

Method for the estimation of renin in tissues. — For the estimation of renin in the kidney, a method based on the formation of hypertensin was developed (25). A small slice of renal tissue (20-30 mg) is carefully minced and extracted with saline (10 mg per ml). Hypertensinase is destroyed according to Leloir *et al.* (23). After centrifuging, a portion of this solution, usually 0.1-0.2 ml is incubated with hypertensinogen * during 2 hours. Hypertensin is estimated by its vasoconstrictor action on a vascular preparation of the toad *Bufo arenarum* Hensel. The standard deviation of this method is 12 %.

Renin activity is usually expressed in units. However, since in these studies we are more concerned with the changes than with the absolute values of renin, we express renin concentration by the amount of hypertensin formed under the conditions of the method employed. The vasoconstrictor action of hypertensin in the problem was compared with that of a standard solution, and its concentration expressed in ml of the standard which gives a similar response.

The place where renin is formed or stored. — The afferent glomerular arteriole shows in its juxtaglomerular portion, replacement of muscular

(*) The hypertensinogen must be free from hypertensinase and renin. We use plasma of nephrectomized dogs as a starting material and the technique employed was that of Dexter, Haynes and Bridges (26).

cells of the medial layer by cells of epithelial character, which are afibrillar and full of granules. The significance of these cells is obscure. It has been suggested by Goormaghtigh ([27]) that they are concerned with the elaboration of renin. Such hypothesis received support from the earlier work of Friedman and Kaplan ([28]) who did not find pressor action in the aglomerular kidney of a marine fish *Poriclythys notatus*. However, it was found out later ([30]), that glomerular kidneys of other marine fish were devoid of pressor substance. Furthermore, the results of Friedman and Kaplan ([29]) in pig fetuses showed that the amount of renin in the kidney bears a relation to the number and size of the renal tubules and not to the development of the glomeruli. The same authors ([31]), attempted to determine more precisely the site of renin formation by destroying the epithelium of the proximal convoluted tube with sodium tartrate. It was found that extracts of those kidneys with tubular necrosis contained almost no renin.

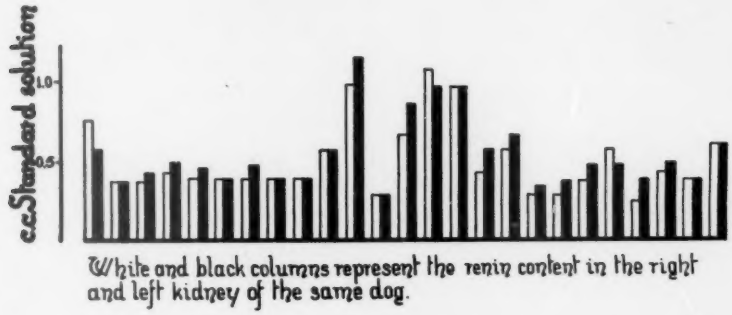
In order to localize the site where renin is stored or formed we took small pieces of tissue from the kidney cortex in different species. Renin was found only in the portion of cortex which lies below the renal capquini, Fasciolo and Fernández Luna, to be published).
sule, to a depth of 2 milimeters or more, according to the animal (Ta-

The renal cortex of dogs presents favourable conditions for a better localization of the site of origin of renin. Here, there is a narrow zone, about ten tubes deep, laying immediately below the capsule completely devoid of glomeruli. This aglomerular zone was found to be rich in renin.

Summarizing, these results show, as previously pointed out by Friedman and Kaplan, that renin is formed in the convoluted tube. The yuxtaglomerular apparatus appears not to be concerned with renin formation.

Renin in normal kidney. — To find out normal variations of the renin content of the kidney, several determinations were made in dogs under ether and seconal anaesthesia ([25]). It was found that the value of both groups agreed fairly well. Nevertheless, some kidneys gave too high or too low values. At present, we cannot say if those results are related to changes in renal circulation or function. However, as shown in figure 1, the renin content of both kidneys of the same dog is strikingly similar, notwithstanding the values being normal, high or low. This is an indication that the renin content of the kidney can be influenced by metabolic or hormonal factors.

Another point of interest is the even distribution of the renin layer in the same kidney. Different samples of subcapsular tissues taken from various parts of the cortex, specially the superior and inferior pole, gave remarkably similar values. This is important because it is possible to tell, at least in the normal animal, from a small piece of renal tissue, if the renin content of the kidney is normal, high or low.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White and black columns represent the renin content in the right and left kidney of the same dog.

FIG. 1. — The figure shows that the renin content of both kidneys of the same dog is nearly the same, although in some cases the pair value is too high or too low.

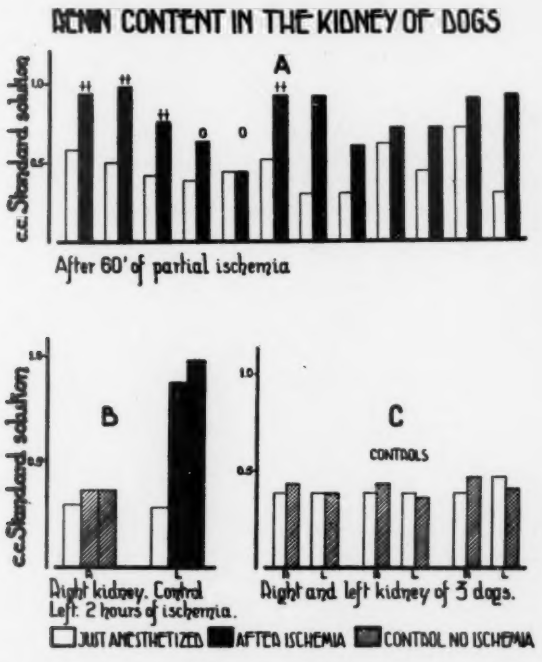

FIG. 2. — A) Shows the increment of the renin content after 60 min. of severe partial ischemia (black columns). The crosses indicate that the renin content of the arterial blood has increased after ischemia. 0 no increase. In the other it has not been investigated.

B) After taking a small sample of renal tissue, the left kidney was severely ischemiated and the right one left without normal blood flow. Two hours later duplicate samples of both kidneys were taken, and their renin content estimated.

C) Renin content of both kidneys after two hours of anaesthesia.

Acute renal ischemia. Incomplete ischemia. — Leloir, Muñoz, Braun-Menéndez and Fasciolo ([3]), observed that if the blood flow through a perfused kidney is reduced, renin appeared in the blood of the renal vein. In these experiments, as in those of Page ([32]) and Dell'Oro and Braun-Menéndez ([10]), the blood flow was severely reduced. It might happen that such an intense ischemia could bring about changes in the capillary permeability allowing the passage of renin into the blood as has been postulated by Bing ([33]). Even though renal ischemia could produce a true secretion of renin, there are at least two possibilities: either ischemia movilizes into the blood the renin stored in the kidney, or besides it is capable of increasing the production of renin.

To study this point dogs weighing 8-10 kg were anaesthetized with seconal ([34]). Immediately after taking a small piece of the kidney cortex, the renal artery was severely constricted. After 60 minutes of ischemia, as shown in figure 2A, the renin content of the kidney was found to be increased in almost every case, with an average increment for this series of 73 %. Control dogs anaesthetized with seconal showed no important changes (fig. 2G.). In one experiment the artery of the left kidney was severely constricted, while the right kidney was left as a control. Duplicate determination made 2 hours later showed that the renin content of the ischemic kidney suffered a 100 % increase, while the control did not change (fig. 2B). Those results show that ischemia not only produces an increase of renin content of the blood, but also brings about an important increment within the kidney.

To investigate how soon incomplete renal ischemia raises the renin content of the kidney, renal samples were taken 5, 15 and 30 minutes after the onset of the ischemia. In some cases renin increased in the first 15 minutes. In the remainder a renin increment was detected at 30 minutes.

Complete ischemia. — Re-establishment of the renal circulation through the kidney maintained in total ischemia by ligation of the pedicle produces a rise of blood pressure ([35, 36, 37]). The pressor effect was ascribed to renin by Prinzmetal, Lewis and Leo ([38]), which was confirmed by Taquini and Braun-Menéndez ([4]).

To find out if total ischemia raises the renin content of the kidney, dogs under seconal anaesthesia were employed ([39]). Samples of renal cortex were taken from both kidneys. The renal pedicle of the left kidney was clamped with an hemostatic forceps, while the pedicle of the right kidney was tied and cut. This extirpated kidney was left loose in the abdomen in order to keep its temperature around 37° C. After two hours, new samples were taken from both kidneys. The results (fig. 3) were surprising. The left kidney showed an average increase of its renin content of 80 %, while no changes were found in the right kidney, whose pedicle had been cut. The increment in the renin content of the kidney with clamped pedicle is of the same magnitude as that found in the partially ischemic kidney, as previously referred. However it is well to remember that in incomplete ischemia the blood which perfuses the kidney removes renin from it, so that the rate of renin formation is apt to be greater in the case of the partially ischemic kidney.

The behaviour of the kidney whose pedicle has been cut is puzzling. No doubt it is, like the one with the clamped pedicle, a totally ischemic kidney, and the only difference between them lies in the fact that in the latter the continuity of the structures has been preserved. This would indicate that a nervous mechanism is acting. It is possible that the forceps would allow impulses to be conducted through the nerves of

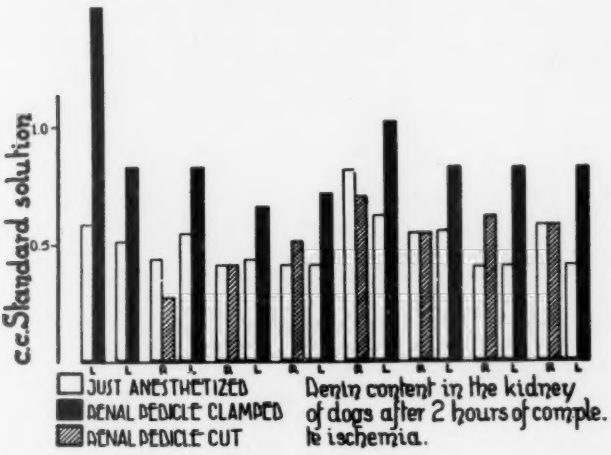

FIG. 3. — The figure shows an increase in the renin content of the kidney whose pedicle has been clamped, and none in the kidney whose pedicle was cut.

the pedicle. As a matter of fact, the compression itself may act as a stimulus. Moreover, it has been observed that the electric stimulation of the renal nerves brings about an increase in the renin content of the kidney (Fasciolo and Taquini, unpublished). But since the stimulation of the renal nerves produces, as is well known, an important reduction of the renal blood flow, it is difficult to ascribe the results of the experiments to a purely nervous action.

The results of Introzzi *et al.*(40), indicate a relation between renal nerves and renin content. They found that after denervation the renin content of the kidney was low in many cases.

As a working hypothesis, to explain the results of the experiments with totally ischemic kidney, let us suppose that the renin content of the kidney can be increased by two mechanisms: a) by the action of incomplete ischemia, and b) by nervous action. Total ischemia would be of no effect and the results obtained when the renal pedicle was clamped, would be due to nervous action.

NEW FIELD OF RESEARCH

The results of the already mentioned experiments show that the amount of renin in the kidney can increase and even be duplicated by 15-60 minutes of renal ischemia. Since renin is a globulin its molecular weight is high and its molecule extremely complex. It seems reasonable to assume that renin exists in the kidney partially under the form of an inactive precursor, pre-renin, which is transformed to renin by ischemia. Williams, Grollman and Harrison (41), believe that all the renin which exists in the kidney is in an inactive form and is spontaneously activated during the process of preparation. We have not observed changes in the renin content when the kidney is extirpated and left for hours at room temperature and even at 37°C. Neither have we been able to observe variations in the activity of the renin solution before or after destruction of hypertensinase. Naturally, it is possible that renin can be activated during the process of estimation, such as trituration of the kidney, destruction of hypertensinase or incubation. At any rate, we have not found evidence of spontaneous activation.

The mechanism by which the ischemia produces the transformation of pre-renin in renin can be investigated. One of the obvious factors is anoxia. In a series of experiments (Taquini and Fasciolo, unpublished) we tried to find if anoxia could produce an increment of renin within the kidney. Dogs were made to breath low oxygen mixtures (about 8 %) but no increase in the renin content of the kidney could be detected after 1 or 2 hours. These experiments do not appear conclusive because the degree of anoxia caused by ischemia was undoubtedly more intense than the one obtained by low oxygen. This point needs reinvestigacion employing other methods such as perfusion, tissue slices, etc.

A certain parallelism seems to exist between the results of determination of renin in blood and in the kidney under different circumstances. An increase of both is found in acute renal ischemia, incomplete or complete; in the latter case renin appears in blood when the clamp of the pedicle is loosened (4). During shock it increases in the blood (5, 6, 7, 8) and in the kidney (40). Respiration of low oxygen mixtures has produced no increases of renin either in blood (5) or in the kidney, as has already been mentioned. It seems to us that it would be of interest to investigate whether some reducing substances such as cysteine, are capable of causing the conversion of pre-renin in renin.

The renin content of the kidney in experimental renal hypertension has been the object of many studies. Some authors (42, 43, 44, 45, 46) found an increase, others a decrease (45, 47). Pickering and Prinzmetal (48) could not observe significant changes. We believe that these studies can be criticised under two aspects. First, the technique employed. Renin has been estimated by making crude extracts of the kidneys and comparing the pressor responses when injected intravenously in an animal. We have already mentioned the shortcomings of the direct method; these are aggravated in this instance because of the use of insufficiently purified extracts, which generally have a strong depressor action.

Second, the duration of the hypertension. It appears probable, on account of the results already mentioned, that in acute hypertension the renin content in the kidney increases. Instead, in chronic hypertension, without increase of renin in blood, this may not be the case. Pickering, Prinzmetal and Kelsall ([49]) found increase of the renin content of the kidney of rabbits with sub-acute hypertension. In chronic hypertension the content of renin was normal.

In a series of 5 dogs with chronic hypertension, according to Goldblatt (Taquini and Fasciolo, unpublished) the estimation of renin in the kidney gave normal values in two cases and low values in the other three. We believe that these experiments should be repeated employing a greater number of animals and estimating the content of renin in the kidney during different stages of the hypertension.

A curious fact observed by Wakerlin ([50]) is the diminution of the renin content in the remaining kidney after nephrectomy of the opposite. He also found in unilateral ischemia, that the renin content in the intact contralateral kidney was frequently low. With Braun-Menéndez we have accomplished a series of renin determinations in the kidney of rats after unilateral nephrectomy (unpublished). It was found that in many cases, but not in all, the renin content was very low.

A JOB FOR RENIN

What is the function of renin? The author is aware of the fact that this question has a teleological meaning and should not be asked. But as no obvious function for renin has been disclosed we cannot resist the temptation to speculate on its function in the organism.

It has been supposed that the kidney is a gland of internal secretion participating in the mechanism which regulates arterial pressure, and that renin is one of the hormones employed by the body in the maintenance of its homeostasis. This hypothesis is supported by the fact that renin is found in the blood of normal animals ([13, 15]) and that its concentration is increased during shock. In this case the increase can be looked upon as an effort of the organism to elevate the blood pressure. Nevertheless Braun-Menéndez and von Euler, ([51]) showed that bilateral nephrectomy not only does not produce lowering of arterial pressure, but that it can produce an increase, which may be even more evident if the life of the animal is prolonged by suitable means ([52]). Moreover, in irreversible shock the time of survival of nephrectomized animals is similar to that of the controls, which indicates lack of action of renin in these cases ([53]).

Let us now assume that renin is concerned with the mechanism which regulates the blood flow through the kidney and with the maintenance of a glomerular pressure adequate to the needs of glomerular filtration. When the pressure in the capillaries of the glomerule falls, which occurs when the renal artery is compressed or in shock, renin produces an increase of the arterial pressure and a vasoconstriction of the efferent glomerular arteriole mechanisms which tend to raise the glomerular pressure. The arterial hypertension could be considered in this case as a compensatory

mechanism for the circulatory insufficiency of the kidney. However, to be able to assign this role to renin, it would be necessary to have proofs of its participation in chronic renal arterial hypertension. As said before, those proofs have not yet been presented.

Apart from its pressor activity, renin is able to modify the excretion of water and salts by the kidney. Pickering and Prinzmetal [54] have shown that small amounts of renin inhibit the diuresis in the rabbit, but greater quantities increase the amount of urine formed. These results have been recently confirmed by Brandt and Gruhn [55], who found that renin in quantities which produce a pressor action gives rise to a marked diuresis with inhibition of the tubular reabsorption of water, sodium and chloride. A curious observation was the fact that proteinuria was produced, not depending of an alteration of the glomerular wall, but due to a defficient reabsorption of protein by the tube.

If renin is involved in the complicated mechanism of water and electrolyte regulation, its vascular action could be presented under a new aspect. Trueta *et al.* [56] have suggested that some modifications of the renal function could be produced through circulatory changes within the kidney.

In this line of thought, if renin is concerned with the metabolism of water and salts, its action might be due to its vasoconstrictor activity, which would produce changes in the blood flow of different portions of the nephron. For instance, its known action on the efferent glomerular arteriole, by increasing glomerular filtration, would increase the amount of water and salt offered to the tube.

The vascular action of renin within the kidney would consequently be linked to its "function" in the regulation of the electrolyte balance. The increase or decrease of the amount of renin present in renal tissue may show therefore, changes related more to this activity than to its circulatory activity.

This interpretation of the function of renin is naturally premature but the author believes that its possibilities should be explored. We should not forget that sodium chloride and the substances concerned with its metabolism (as for instance, desoxycorticosterone) have been shown to be capable of producing or aggravating renal arterial hypertension.

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THE DOMESTIC CAT AS A LABORATORY ANIMAL FOR EXPERIMENTAL NUTRITION STUDIES

I. General considerations, care and feeding of the animals

BY ALBERTO CARVALHO DA SILVA

(Department of Physiology (*) Faculdade de Medicina Universidade de S. Paulo — Brasil)

WITH EXCEPTION of the report by Krehl and Welt (1948), no systematic work on the nutritional requirements of the domestic cat has been published. However this animal would be convenient for the biochemical and hematological study of the nutrition deficiencies because it resists considerable venous bleeding at frequent intervals and is much more economical to maintain than the dog and the monkey.

As a first step in this new field I decided therefore to study the problem of raising the cat on purified rations under standardized experimental conditions. I have been successful in this attempt and also in obtaining experimental deficiencies in vitamins as will be reported in subsequent papers. In this first report details are given about care and handling.

Source and selection of the animals. — The most convenient age for the beginning of the experimental period is 8 to 9 weeks with an average weight of 700 g for males and 600 g for females. The animals can be brought to the laboratory at the proper age, or else the mother with the recently born litter can be placed in a large wooden cage. Raw beef liver, raw beef muscle, fish and milk, are given daily. Kittens showing the diseases referred to below, are not accepted.

Attempts to have a reproduction colony were unsuccessful. Pregnancy occured sometimes in caged animals in stock rations (see below) but the kittens died within the first days. No pregnancies were observed on purified rations although the males exhibit sexual interest when about one year old.

Cages. — The cages used for nutrition work are of cubic shape, about 60 cm of side. Walls are of wire screen; the floor is also of wire screen but large enough to let the feces fall through directly on the

(*) Head Prof. F. A. de Moura Campos.

animal room floor. As some of the animals on purified rations get used to defecate in the food container this should be fixed to the cage wall. At the beginning some of the experiments were carried out with wooden bottom cages and no difference in growth rate was observed. Although real coprophagy was observed only once in this non screened cages, food and water are easily contaminated with feces.

Room temperature is always maintained above 23° or 24°C because kittens, especially when on purified rations are extremely sensitive to temperature fall and air currents.

Feeding. — Detailed composition of the purified rations will be given in the following paper. They should be supplied as a soft paste. This can be obtained dissolving 10 or 20 g of purified gelatin in water and adding this solution to 1 kg of the dry rations. The proper amount of water to be used depends on the composition of the ration. For 1 kg containing 35 % of Labco casein, 500 ml is satisfactory. When filter paper is added, a pulp should previously be prepared in the blender with a part of the water to be used.

Three stock rations were used with equally good results as far as growth, blood picture and general appearance of the animals are concerned (table I).

TABLE I

Composition of stock rations used (Supplied as a paste including the water used on cooking)

Ingredients	*Ration A*	*Ration B*	*Ration C*
	per cent	*per cent*	*per cent*
Crude casein	10.0	10.0	—
Dry whole milk	20.0	—	—
Raw beef liver	35.0	—	—
Raw beef muscle	—	20.0	33.3
Steamed deboned and eviscerated sardines	—	20.0	33.3
Compressed oats slightly cooked	30.0	20.0	—
Cooked and mashed potatoes	—	15.0	33.3
Butter, lard or vegetable oil	5.0	10.0	—
Bone meal	—	2.0	—
Cod liver oil	—	3.0	—
	100.0	100.0	100.0

When large volumes have to be forcefed (tomato juice, vermifuges, etc.) a rubber tube as used on blood pipets, is a convenient stomach tube. Small supplements can be placed in the mouth with a syringe. If the animal dislikes the taste (B vitamins solution, aureomycin) the supplement can be emulsified with 2 ml of cotton seed oil.

Bleeding. — As much as 40 ml of blood can be obtained at one time from the saphena at the internal face of the thigh, after clipping the

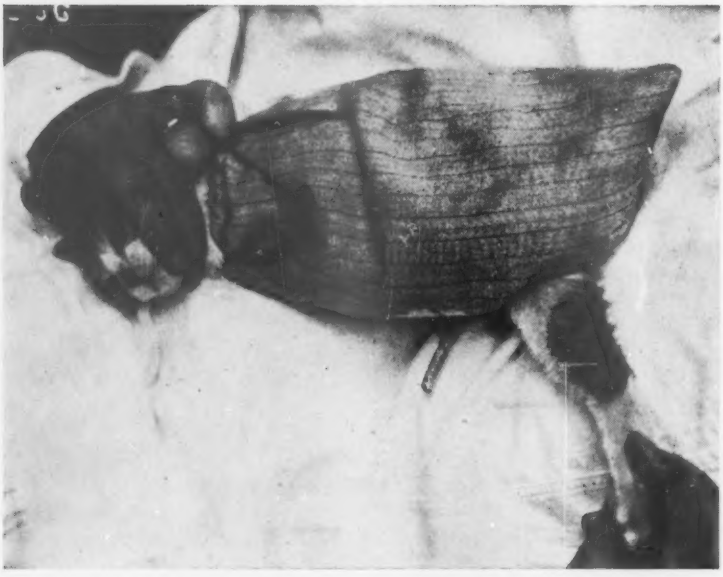

FIG. 1 — A convenient way of holding the animal for bleeding work.

fur and washing with alcohol to improve the visibility of the vein. For blood counts, excitation of the animal must be carefuly avoided because values for hemoglobin and red cells might be too high. For bleeding work in general, one may find it convenient to place the animal in a cloth bag as shown in fig. 1. Local anaesthesia with 2% novocain may be used but is seldom required.

Deworming. — As most of the animals coming to the laboratory are infested with *Isospora Felix* and *Ascaris Toxocara* 3 to 5 drops of chenopodium in 5 ml of ricinum oil may be given by stomach tube after 12 hours of fasting. Usually 4 to 8 worms are passed during the following 12 hours but as much as 37 worms were eliminated by one animal.

Diseases. — A few diseases were found to interfere significantly with the experiment, at least in my laboratory.

1) A skin mycosis caused by a *microsporum* is very common especially on kittens. Although it is rather benign in well nourished free animals and also in most of those caged over in stock rations, it is more serious and extremely difficult to control in animals on purified diets. Extensive areas of the body may be involved; contagion is very easy and

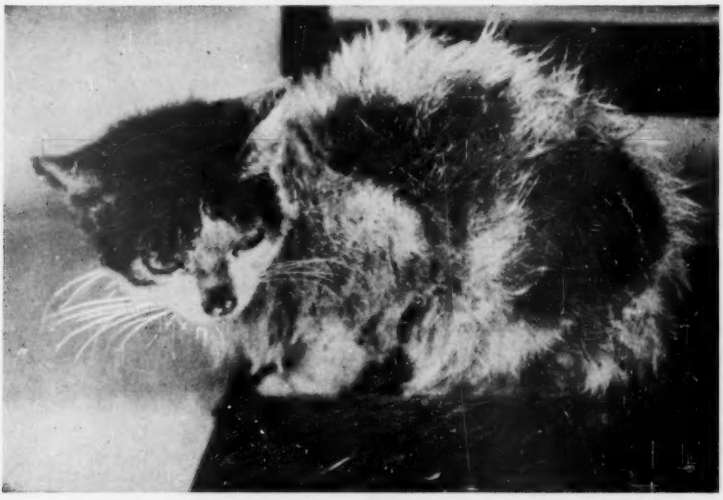

Fig. 2

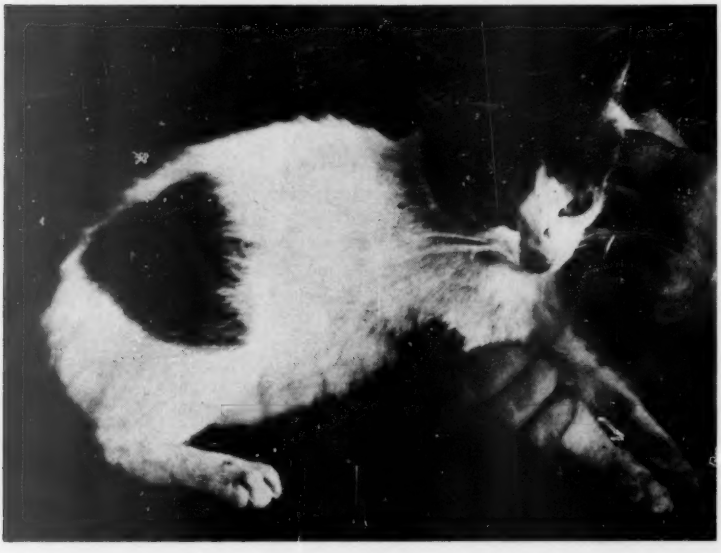

Fig. 3

FIGS. 2 AND 3 — Cat nº 82; "microsporiasis" on purified ration (fig. 2) and 20 days after being transferred to stock ration B (fig. 3).

extremely difficult to avoid. No treatment was found satisfactory for animals on purified rations. A talcum containing undecilenic acid and sodium propionate can be spread at frequent intervals on the animals and the cages to control the progress of the disease. A lotion with the same ingredients is convenient for topical applications. Complete recoveries were observed by transferring very sick animals to stock rations (figs. 2 and 3). As a strict rule, any animal showing even the slightest loss of hairs or a dandruff-like aspect of the fur, or any animal suspected of previous contact with a sick animal, should never be admitted to the laboratory.

2) The well known leucopenic disease of the cat was observed once, exhibiting high mortality and epidemic character. One should be very well informed about it when conducting nutrition work because the association of weight loss and leucopenia may simulate a deficiency, especially of folic acid. As in spontaneous recovering animals leucopenia is rapidly followed by leucocytosis and as weight resuming is dramatic, the simulation of "deficiency" is complete when the "supplement" is tried at the strategic moment. For a very good and detailed study see Hammon *et al* (1939), Syverton *et al.*(1943), and Levaiditti *et al.*(1943).

3) In my experience, however, the main difficulty is an extremely contagious disease of the respiratory tract, characterized by loss of weight, ocular and nasal secretion, and more or less pronounced sneezing and cough. Mortality is high especially on purified rations, and occurs after 6 to 15 days of illness. More than half of the animals placed on experiment were lost or discarded for this reason. From 16 kittens placed on purified diet with sulfaguanidine, without niacin and folic acid, only one survived. Extensive areas of pulmonitis are frequently found, if the animal has been sick long enough. Leucocytes are high (around 30 000). Surviving animals on purified rations frequently fail to resume normal growth rate for as long as 20 days, simulating a deficiency status. Therefore one must be extremely careful with the interpretation of supplementing effect especially because symptoms of the acute period are occasionally mild. However, if animals surviving the acute period on purified rations are placed on stock rations, recovery is very pronounced.

Numerous attempts have been made to identify the causative agent. A very similar disease was described by Baker (1943) but up to now, I have been unable to collect evidence for the presence of the "Baker's virus". This suggests a possible relation with the disease reported by Blake *et al.*(1942). Penicillin, aureomycin, and sulfa-drugs were ineffective during the acute period. However penicillin (10 000 units, 4 times a day for five or six days) has a favourable effect during the convalescence period. High doses of ascorbic acid were tested but no certain conclusions could be drawn *. Surviving animals exhibit an appreciable resistance to reinfection for a long period.

(*) Accumulated observations suggest that on purified rations lacking niacin the kittens always develop the disease with a very malignant character.

Aknowledgement

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Mrs. Carvalho da Silva was willing to carry a large part of the routine work. The dedication of Mr. Miguel dos Santos, Mr. Urben Eugenio dos Santos and Mr. Alexandre José da Silva, employees at the laboratory, is also gratefuly aknowledged.

SUMMARY

Care and handling of the domestic cat for nutrition experiments are described. Reference is made to interfering diseases.

SUMARIO

Se describen los métodos y los cuidados de mantenimiento del gato para experimentos sobre nutrición. Se mencionan algunas enfermedades que interfieren en el curso y la interpretación de experimentos de este tipo.

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THE DOMESTIC CAT AS A LABORATORY ANIMAL FOR EXPERIMENTAL NUTRITION STUDIES

II. Comparative growth rate and hematology on stock and purified rations

ALBERTO CARVALHO DA SILVA

(Department of Physiology (*) Faculdade de Medicina. Universidade de S. Paulo — Brasil)

STOCK RATIONS, care and handling of the animals are described in the preceding publication (Carvalho da Silva, 1950). Two purified rations were used through the experiment now reported (table I).

Labco Vitamin free casein was used. For short periods however, an "acid washed" purified casein, prepared at the laboratory according to the technique of Cannon *et al.* (1945) was used.

Samples for blood counts were obtained as reported before. Hemoglobin was determined with the Evelyn photoelectric colorimeter (Evelyn, 1936) calibrated against the manometric method for oxygen capacity (Van Slyke, 1932). For hematocrits 10 cm long capillary tubes of the type used in 0.1 ml pipets were sealed at one end with sealing wax, and centrifuged at 3 000 r.p.m. for half an hour. Blood should be carefuly mixed because sedimentation rate is sometimes extremely high.

RESULTS

Growth results are summarized in table II. Although more than sixty cats were used in this experiment, many had to be discarded in view of intercurrent diseases (see preceding publication).

The results for the first 12 weeks of experiment on stock rations and on purified ration 1 are plotted in fig. 1. As weights were recorded daily during this period, the standard deviation was calculated for animals in purified ration 1 according to the formula

$$S = \sqrt{\frac{n \Sigma x^2 - (\Sigma x)^{2**}}{n(n-1)}}$$ and are reproduced in fig. 2 for males

and in fig. 3 for females. Only one male was a little over 2 S during a short period. All the other animals were comprised between the intervals + 2 S and — 2 S.

Hematological data are summarized in table III.

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* Head Prof. F. A. de Moura Campos.

** As suggested by Prof. Pedro Egydio de Carvalho, Fac. de Higiene. Univ. de S. Paulo.

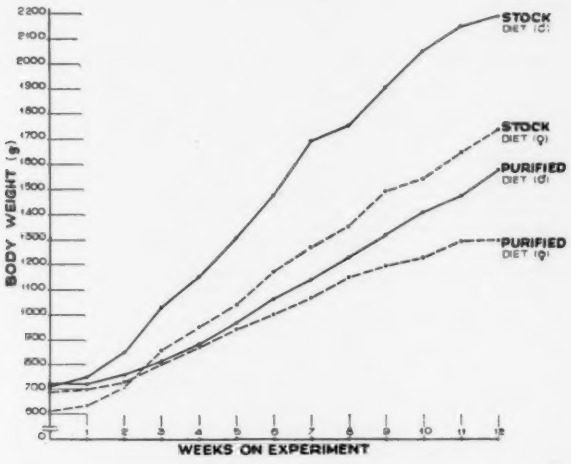

FIG. 1. — Growth rate of cats on stock rations and purified ration "1" for males and females, during the first 12 weeks of experiments.

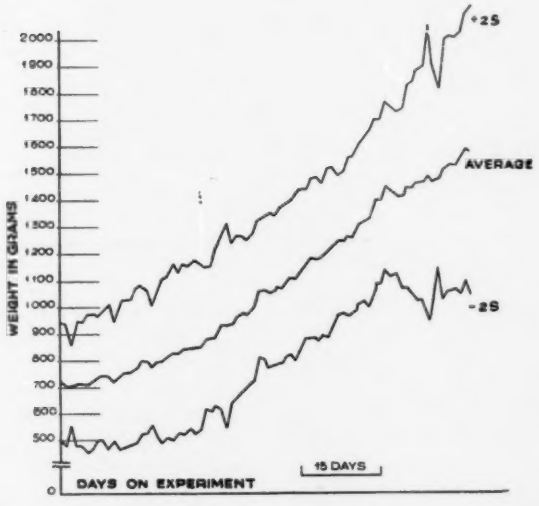

FIG. 2. — Average daily growth rate for male cats on purified ration "1"; upper line: average + 2S; lower line: average — 2S.

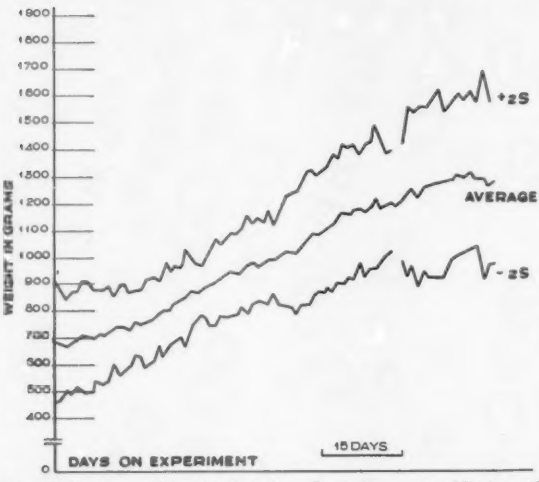

FIG. 3. — Average daily growth rate for female cats on purified ration "1"; upper line: average + 2S; lower line: average — 2S.

TABLE I

Composition of purified rations *

	Ration 1 per cent	Ration 2 per cent
Vitamin free casein	35	35
Purified gelatin	1	1
Sugar	33	33
Butter	—	25
Lard	12.5	—
Cotton seed oil	12.5	—
Salts IV (Phillips-Hart, 1935)	5	5
Filter paper or "Ruffex"***	1	1

VITAMINS **

	mg		mg
Thiamine	0.75	Calcium pantothenate	2.5
Riboflavin	0.75	Nicotinic acid	2.5
Pyridoxine	0.75	Folic acid	0.5
Para amino benzoic acid	0.75	Biotin	0.05
Vit. K (2-4 dimethyl naphtoquinone)	0.75	Inositol	30.0
		Choline	300.0
		Alpha-tocopherol	15.0

* Details for the preparation of the purified rations are given in the preceding publication (Carvalho da Silva, 1950).

** Amounts given three times a week, by mouth, emulsified with 2 ml of cod-liver oil.

*** Eimer and Amend, New York.

TABLE II

Growth rate on stock and purified rations. Age at the beginning of the experiment. 60 days. The figures in parenthesis indicate the number of animals averaged.

Weeks on experiment	Stock ration		Purified rations		
			Ration I		Ration 2
	males	females	males	females	males
	g	g	g	g	g
1	710 (6)	606 (7)	706 (17)	671 (10)	845 (2)
2	752	638	736	705	800
3	852	717	779	765	895
4	1029	865	835	842	910
5	1158	947	907	900	972
6	1309	1045 (5)	1009	986	1085
7	1473	1173 (6)	1064	1047	1160
8	1693	1253 (7)	1163	1128	1255
9	1757	1345	1292	1209	1245
10	1908	1494 (4)	1369	1258	1360
11	2045	1545 (7)	1433	1271 (9)	1440
12	2149	1651	1595 (12)	1336	1355
13	2184	1737 (4)	1624	1404 (7)	1435
14	2335	1803	1709 (11)	1499 (5)	1465
15	2458	1702	1901 (4)	1619	1610
16	2469	1749	1802	1660 (4)	1690
20	2764	2020	2247	1855	1930
24	3935 (5)	2320 (2)	2288	2024	1885
28	3190	2303 (3)	2648 (3)	2327	
32	3274	2500	2774	2590	
36	3264	2660 (2)	2930 (2)		
40	3272	2755	3120		
44	3275 (4)	2607	3247		
48	3480	2590	3420		
52	3553 (3)	2657	3665		
56	3503	2653	3895		

Discussion and conclusions

The reported data indicate that there is no difference in the weight of animals raised on stock and purified rations at least on ration 1. Red cells, hemoglobin concentration and hematocrit seem to be a little lower on purified diets, with a slight tendency for macrocytosis. However they are identical during the first five months. In my experience, this constitutes a sufficiently long period to study vitamin deficiencies on the cat.

The difference in growth rate on stock and purified rations for the first months of experiment is very pronounced. Attempts have been made to correct this difference and they will be published in

TABLE III

Comparative blood counts of male and female cats at different ages on stock and purified rations

Ration	Days on experimen.	Sex	No. of counts	No. of animals	Ht		Hb		R. B. C.		W. B. C.		M. C. V.		M. C. H.		M. C. H. C.	
					Aver.	S	Aver.	S	Aver.	S	Aver.	S	Aver.	S	Aver.	S	Aver.	S
Purifield	8-60	m.	11	9	35.1	4.2	11.7	1.6	7.87	1.30	17.3	3.1	49.2	5.7	16.4	3.0	33.9	2.4
		f.	12	9	37.7	3.7	13.0	0.8	7.09	1.10	15.3	3.4	52.7	10.3	17.2	3.7	31.5	3.0
	80-140	m.	13	9	36.5	3.0	12.2	1.3	7.15	1.32	15.3	5.5	55.1	10.9	17.3	2.7	32.7	1.1
		f.	6	4	34.2	2.9	11.9	0.7	6.40	1.36	13.0	4.3	56.5	10.3	19.2	3.7	34.8	3.3
	adults	m.	9	5	41.1	4.7	13.3	1.2	8.54	0.82	16.4	5.6	47.8	3.8	15.2	1.1	32.3	1.2
	(140-460)	f.	6	4	38.7	3.2	12.5	1.5	7.69	1.45	18.8	10.7	51.3	6.6	16.6	2.5	32.5	1.5
Stock	0-140	m.	11	9	37.5	5.9	11.7	1.4	7.73	1.53	15.8	5.9	49.3	7.1	15.4	2.6	34.1	2.4
		f.	13	12	39.6	5.6	12.4	1.8	8.17	1.84	19.9	7.8	49.3	6.7	15.3	1.9	31.1	2.7
	adults	m.	10	7	46.0	3.7	15.2	1.1	10.27	1.21	14.1	1.3	44.5	4.3	14.7	1.6	33.3	1.1
	(140-425)	f.	6	4	43.0	4.1	15.0	1.9	9.30	1.90	14.3	4.0	47.3	5.9	16.3	1.4	34.3	2.3

Ht.: hematocrit; Hb.: hemoglobin in g per 100 ml; R. B. C.: millions of red cells per c.mm; W. B. C.: thousands of white cells per c.mm.; M. C.V.: mean corpuscular volume; M. C. H.: mean corpuscular hemoglobin; M. C. H. C.: mean corpuscular hemoglobin concentration. For the meaning of these indices see Wintrobe (1947).

The same formula mentioned in the text was used to calculate "S". As on some animals two counts were recorded the "no. of counts" is larger than the "no. of animals". All the animals placed on experiment at 60 days of age.

detail. It is, however, the present impression of the writer, that ascorbic acid, vitamin B_{12}, liver extract (Reticulogen), raw sardine, brewers, yeast, tomato juice, are not effective for improving growth. Supplementation experiments are frequently complicated by the large individual variation and diseases.

However, it is evident that if the purified rations used lack of some nutritive factor for the cat, this is not a fundamental one. It should be emphasized that the best animals on purified rations exhibited a better growth rate from the beginning than the worst animals on stock rations. As a matter of fact the maximum weight observed belongs to a male on purified ration 1, that attained 5 kg at 14 months of age. Krehl *et al* (1948) say that a purified ration based on the composition of the cow's milk does not support good growth for cats, but a normal growth is obtained when the proportions of the bitch's milk are adopted. As my purified rations are based on the composition of the cow's milk my results do not support their conclusions. However, as they did not publish their values, no comparison is possible. Furthermore, it should be kept in mind that the cat exhibits a highly developed and selective appetite. Pronounced modifications are observed on growth rate and food consumption by simply modifying the consistency of the ration.

Although most of the animals reported now received butter as a source of fat, I have been using lard plus cotton seed oil for periods as long as 10 months, with the impression that the result is the same as with butter at least for adult animals. The two males included in table II are still in good health after 10 months on experiment. It seems, however, that growth rate is lower than on butter.

It should be emphasized that some of the animals here reported were kept on purified ration for other experiments up to the age of 700 days and maintained in good health even when 0.5 % of sulfaguanidine was added to the ration.

In view of the reported results, it seems acceptable to conclude that the domestic cat can be used in nutrition research, with the advantage of offering more facilities than the rat to study biochemical and hematological aspects of deficiencies. This conclusion is reinforced by the fact that I was able to obtain folic acid, pantothenic acid, pyridoxine deficiencies, as will be reported on subsequent publications. However, one should take special precautions and have a satisfactory training with this animal on this type of experiments, to avoid faulty interpretations.

Aknowledgement

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SUMMARY

Comparative growth rate and hematology of the cat, on stock and purified rations were studied up to one year of age. Results prove that, on a purified ration with a composition approximated to the cow's milk the known growth factors are satisfactory, although growth rate is slower on purified rations.

SUMARIO

O ritmo de crescimento e quadro hematológico do gato são comparativamente estudados en racões naturais, até á idade de um ano. Os resultados provam que em uma ração pura, de composição aproximada ao leite de vaca, os fatores de crescimento conhecidos são suficientes, embora o ritmo de crescimento seja mais lento.

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RENOTROPHIC ACTION OF LEAD IN THE RAT

BY HUGO CHIODI AND RODOLFO SAMMARTINO

(Instituto de Biología y Medicina Experimental and Instituto de Anatomía Patológica "Telémaco Susini"-Buenos Aires-Argentina)

THE EFFECT of lead poisoning on the kidney is still a matter of controversy. Very many clinical and experimental works on the subject have been published and the results obtained may be classified in three groups: a) lead only affects certain zones of the renal tubules without inducing vascular or glomerular lesions, except that in some cases a secondary sclerosis ("glandular cirrhosis" of Charcot) may appear; b) lead is a vascular poison producing glomerular lesions and a primitive renal sclerosis; c) lesions caused by lead may be composite: tubular and vascular, though the latter appears only after a certain time. For a review of the literature the book of Cantarow and Trumper (1) "Lead poisoning" should be consulted.

Calvery *et al.* and Finner and Calvery (2, 3) observed an increase in size of the kidney of dogs and rats chronically poisoned with lead acetate, which was given to these animals mixed with their food. In rats poisoned during 6 to 8 months, the kidney was 22 per cent greater than in the controls. The epithelial cells of the tubules were irregular in size with occasionally greatly hypertrophied nucleii containing sometimes acidophilic bodies. These alterations were more marked in the medullary zone but were also present in lesser grade in the cortex. Inflammatory reaction or necrosis were not observed. The renal corpuscles and the vessels were free of lesions. The authors consider the increased weight of the kidneys as due to hypertrophy of the tubular epithelium.

Fairhall and Miller (4) describe similar lesions in rats poisoned during 2 years with lead carbonate.

Chiodi and Sammartino (5) observed greater renal hypertrophy than that described by Calvery *et al.* in rats poisoned with lead acetate given by stomach tube.

MATERIAL AND METHODS

White rats raised in the Instituto de Biología y Medicina Experimental were used. The animals were fed the following diet:

Milk	64	per cent			
Bread	20	„			
Corn	8	„	„		
Wheat	8	„	„		
Yeast	0.2	„	„	once a week	
Cod liver oil	0.08	„	„	„	„

In one of the experiments a diet rich in protein was given with the following composition:

Casein	30	per cent
Wheat flower	30	„ „
Corn flower	29	„ „
Yeast	5	„ „
Cod liver oil	5	„ „
Salt mixture (Mc Collum)	1	„ „

Lead poisoning was produced by giving daily, by stomach tube, 2 ml of a 0.5 to 12 per cent solution of lead acetate in distilled water.

Thyroid was administered as a dry powder of bovine thyroid gland at a dose of 20 mg per day by means of a small spoon. Testosterone propionate (Perandren) in oil was injected subcutaneously in daily dose of 1 and 2.5 mg.

Inulin clearance and diodrast Tm were determined using the method of Braun-Menéndez and Chiodi ([6]).

BAL (dimercaptopropanol) was injected intraperitoneally in water. Each day 66 mg per kg weight were given, divided in two doses.

Blood pressure was measured by the method of Williams, Harrison and Grollman ([8]). Values between 80 and 120 mm|Hg were considered as normal.

Results

Action of lead acetate on the weight and microscopical structure of the kidney.

Weight. — As shown in table I, the administration of lead acetate caused a definite increase in the kidney absolute weight, as well as in the relation kidney weight|100 cm^2 body surface. This increase in weight was already present 10 days after the initiation of lead acetate administration and persisted for 12 months. No evident proportionality seemed to exist between kidney weight increase and daily dose of lead acetate: medium doses (4.5 per cent) had a renotrophic action very similar to greater doses (6, 8 and 12 per cent). A greater kidney growth was observed in females but the reduced number of observations do not allow definite conclusions.

Histologic observations. — Histologic study was made in 36 animals which had received lead acetate in concentrations of 0.5 to 12 per cent during 10 days to 11 months.

Fig. 1 shows a cross-section of the kidney of a rat which received during 4 months lead acetate at a concentration of 4.5 per cent. The density of the renal tissue is greater than in the corresponding control (fig. 2) due to a considerable reduction in the lumen of the renal tubules caused by the hypertrophy of the tubular epithelial cells.

The following microscopic lesions we consider as typical and with slight variations in intensity and extension were found whatever the dose or the time of intoxication:

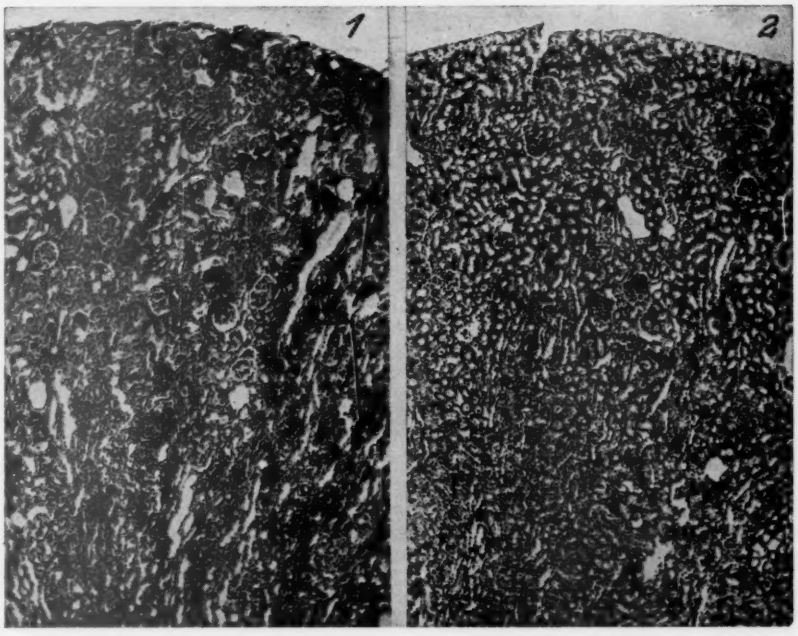

FIG. 1. — *Renal cortex of rat poisoned with 4.5 per cent lead acetate during 4 months (see figs. 7 and 9). Compact aspect due to generalized hypertrophy. Zones (P) with precipitates (Incl. 27189; × 52).*
FIG. 2. — *Renal cortex of normal rat (Incl. 27420; × 52).*

The *renal corpuscles* (fig. 3) are more clearly seen, due to a moderate retraction of the glomerulus and to the existence of a small amount of albuminous exudate of low density in the lumen of the corpuscle. Occasionally partial adhesions of the glomerular tuft to the Bowman's capsule are to be seen. There are therefore, signs of slight glomerulitis.

In the *tubules* lesions localize themselves first and predominantly in the Henle loops with later extension to the proximal convoluted tubules.

Early lesions are characterized by: a) increase of cell size, specially at the expense of the protoplasm which frequently appears more granulous and dense, and shows some basophilism (fig. 4); b) formation of mononucleated giant cells in every zone of the tubules but predominantly in the intermediate zone between cortex and medulla. These are polygo-

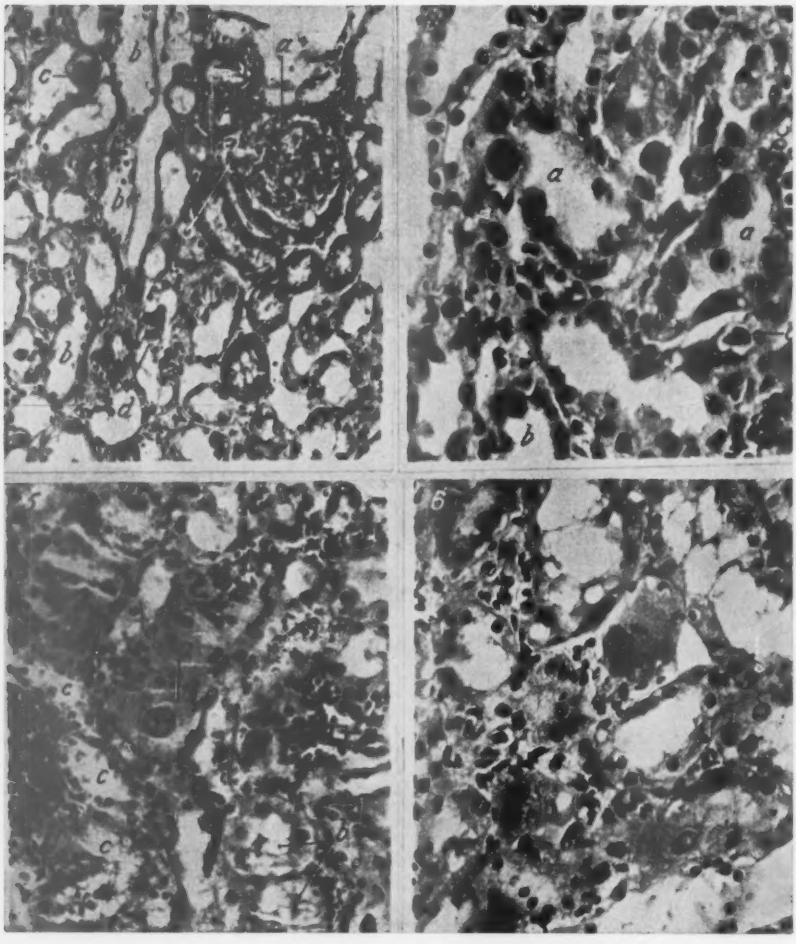

FIG. 3. — *Rat poisoned with 8 per cent lead acetate during 2 months*: a, *retracted glomerulus with albuminous exudate in the corpuscle's lumens;* b, *tubules showing epithelial disgregation;* c, *giant cell;* d, *precipitates in tubular lumen (Incl. 27096;* × *275)*

FIG. 4. — *Rat poisoned with 6 per cent lead acetate during 10 days.* a, a, *convoluted tubules with enormous epithelial hypertrophy when compared with a normal epithelium (b) of Henle loop;* c, *intranuclear body. (Incl. 26987;* × *605).*

FIG. 5. — *Rat poisoned with 12 per cent lead acetate during 3 months.* a, *cell with gigantic hypertrophy;* b, *tubules with vacuolar degeneration;* c, c, c, *tubules with granular disgregation of the epithelium. Pseudosclerosis due to atrophy of paren chyma (*× *325).*

FIG. 6. — *Same animal as in fig. 3. Marked cellular hypertrophy with plasmodial formation (*× *615).*

nal and globulous elements (figs. 3, 4), with a diameter many times greater than that of a normal cell, with proportional increase in size of nucleus and protoplasm. The hypertrophy is almost generalized but variable in different sections. Occasionally mitosis are to be seen even in giant cells. Sometimes groups of hypertrophied cells become confluent in syncisial masses (fig. 6). In some sections the variability of form and size of the nuclei is quite remarkable (fig. 4).

Later on degenerative phenomena are added, characterized by:

a) vacuolar degeneration of some epithelial cells (fig. 6).

b) Frequently granulo-hyaline degeneration with gradual disgregation of the cellular apical zone and elimination of the epithelium; in some sections cavities are seen limited by the hyaline basement membrane (figs. 3, 5).

c) In the hypertrophied nucleii the characteristic alteration described by Blackman (9), Calvery (10) etc. is observed. It consists in the condensation of chromatin in a central cluster surrounded by a kind of circular vacuole, the nuclear membrane being thickened and intensely coloured (fig. 8).

The degenerative and regenerative processes have a variable distribution, being sometimes intermingled in the same tubule.

As soon as 10 days after the initiation of lead administration casts were constantly observed in the lumen of the tubules. They are violet-colored bodies, which appear as amorphous or granular masses occasionally in form of concretions of concentric disposition, frequently surrounded by an albuminoid cover with calcium salts deposits (fig. 7).

The renal alterations described were well established as soon as 10 days after the initiation of the lead acetate administration. From their initial localization in the intermediate zone they extended themselves to the cortex according as the concentration of lead acetate was increased from 0.5 to 4.5 per cent. A greater intensity of the lesions was also observed with the increased dose. Concentrations greater than 4.5 per cent did not cause a proportional increase in the intensity of the lesions.

No appreciable vascular alterations were observed even in preparations stained with special dyes (fig. 8).

In the kidney specimens of rats poisoned during 10 to 30 days no increase in connective tissue was observed. But in animals with 4 or more months of poisoning a moderate increase of the intertubular stroma was to be seen in zones where lesions were most intense with disappearance of the epithelium, thickening of the basement membrane and increase of reticulin (fig. 1, 5, 9). We believe this sclerotic process to be more apparent than real and due simply to the coming into close contact of the walls of the tubules which have lost their epithelium. Proliferative stimulation of the mesenchyma or primitive renal sclerosis were never found.

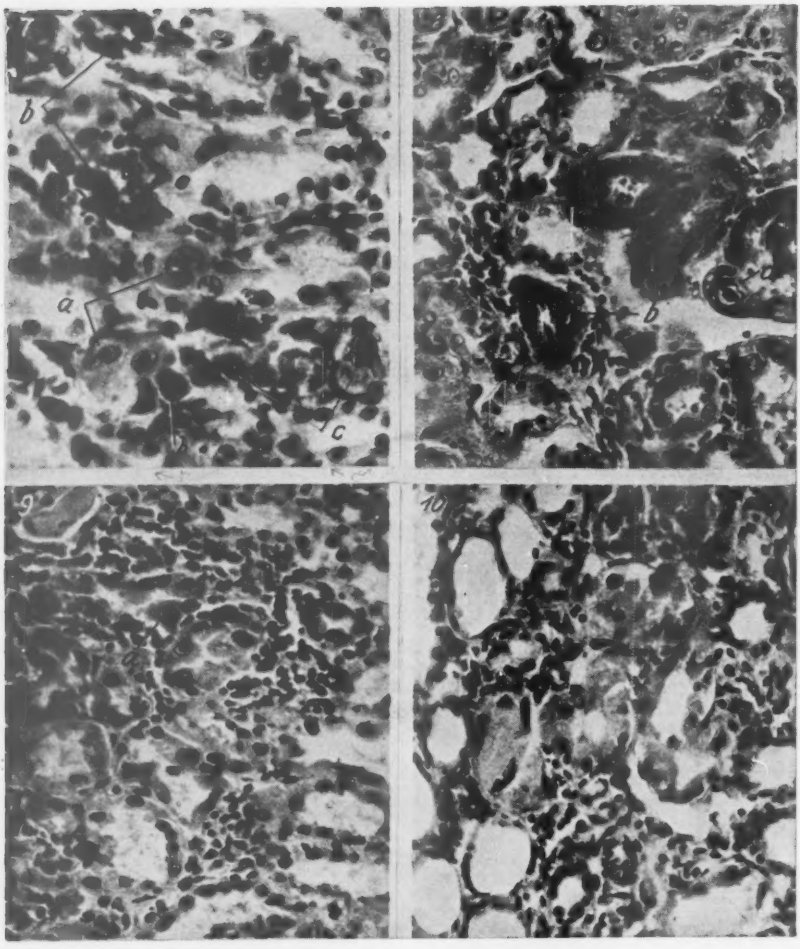

FIG. 7. — *Same animal as in figs. 1 and 9.* a, *cellular hypertrophy;* b, *opaque calcified precipitates;* c, *dense albuminous masses with calcareous impregnation* (× 740).

FIG. 8. — *Rat poisoned with 4.5 per cent lead acetate during 11 months. Convoluted tubular and Henle loops with cellular hypertrophy and basophilism.* a, *giant cell with intranuclear body;* b, *arteriole free of lesions* (× 495).

FIG. 9. — *Same animal as in figs. 1 and 7. Degenerative lesions in some cells, hypertrophy in others.* a, *intranuclear body. Pseudosclerosis due to atrophy of parenchyma.*

FIG. 10. — *Rat poisoned with 4 per cent uranium acetate during 10 days. Diffuse necrobiosis of the tubular epithelium without hypertrophy (Incl. 26979;* × 565).

Action of uranium acetate on the histological structure of the kidney.

Weight. — Due to the small number of animals used in these experiments as well as the high mortality rate produced by the administration of uranium acetate, we could not arrive at definite conclusions as to the kidney weight variation in the poisoned animals. But it may be interesting to mention that in 2 of the 3 animals which survived 18 days the daily administration of 2 ml of a 3 per cent solution of uranium acetate, no marked increase of kidney weight was noted.

Histologic observations. — A histologic study was made in 9 animals which received uranium acetate in concentrations of 0.01 to 6 per cent during 10 to 18 days.

Concentrations of over 0.5 per cent produced evident lesions consisting in: glomerular congestion, granular degeneration of the convoluted tubules with disgregation of the apical zone of the cells and occasionally necrosis of a whole sector, and degenerative changes in the nuclei (fig. 10). Some irregular hypertrophic nucleii were observed without the characteristic alterations of lead poisoning. No casts were seen and only exceptionally a calcareous cylinder. As a whole the lesions were of degenerative and necrobiotic type.

Action of simultaneous administration of renotrophic agents and lead acetate on the weight and histological structure of the kidney.

Weight. — The simultaneous administration of a protein rich diet and the 4.5 per cent solution of lead acetate produced a 91 per cent increase in kidney weight with respect to controls of the same sex and fed the same diet (table II). This weight increase is greater than that observed (75 per cent: see table I) when identical dose of lead is given to rats fed the standard diet. Therefore proteins potentiate the renotrophic action of lead.

Opposite results are obtained when lead acetate is given simultaneously with thyroid powder or testosterone propionate. There is also a summation of renotrophic effects but the effect of lead is less than when administered alone (table II).

Histologic study. — This was made in 11 rats fed the protein rich diet and poisoned with the 4.5 per cent solution of lead acetate, 2 of them during 10 days and the rest during 4 months (table II).

In these animals lesions were of similar type but of greater intensity than those observed in rats fed the standard diet and receiving the same amount of lead acetate. In the lumen of the renal corpuscle an albuminous exudate was frequently seen.

No appreciable lesions were found in the kidneys of the 5 control animals fed a protein rich diet.

A histologic study was made of the kidneys of 2 uninephrectomized rats receiving 1 mg per day of Perandren during 30 days and of 6 rats receiving 2.5 mg per day also during 30 days, all of them poisoned with the 4.5 per cent solution of lead acetate. The lesions observed, when

TABLE I

Influence of dose and time of administration of lead acetate on kidney weight

SERIES	Nº rats	Sex	Body weight g	Kidney weight absolute mg	Kidney weight per 100 cm2 body surface** mg	Percentage increase
Normal controls	24	♂	191 ± 4.5*	1723 ± 40.2	457 ± 7.7	
Normal controls	18	♀	191 ± 9.9	1403 ± 66.3	382 ± 11.5	
Lead acetate 0.5 per cent during 10 days	3	♀	115 ± 4.6	1381 ± 128.0	512 ± 34.8	34
Lead acetate 1.0 per cent during 10 days	3	♀	117 ± 2.9	1478 ± 90.2	543 ± 25.1	42
Lead acetate 2.0 per cent during 10 days	3	♂	198 ± 6.8	2497 ± 163.0	646 ± 12.1	41
Lead acetate 3.0 per cent during 11 days	6	♀	110 ± 4.3	1604 ± 114.5	613 ± 30.7	60
Lead acetate 3.0 per cent during 30 days	7	♂	175 ± 5.1	2163 ± 92.0	606 ± 16.8	33
Lead acetate 4.5 per cent during 9 days	10	♀	114 ± 4.4	1471 ± 61.6	552 ± 18.4	45
Lead acetate 4.5 per cent during 10 days	5	♂	116 ± 5.1	1894 ± 16.2	695 ± 44.0	52
Lead acetate 4.5 per cent during 29 days	5	♂	209 ± 8.9	2726 ± 131.0	683 ± 27.3	49
Lead acetate 4.5 per cent during 60 days	7	♂	212 ± 14.8	2686 ± 156.7	680 ± 23.8	49
Lead acetate 4.5 per cent during 120 days	6	♀	159 ± 2.4	2224 ± 52.7	667 ± 18.2	75
Lead acetate 4.5 per cent during 12 months	7	♀	190 ± 10.4	2322 ± 148.5	653 ± 34.3	71
Lead acetate 6.0 per cent during 60 days	5	♂	194 ± 9.6	2380 ± 110.8	630 ± 22.5	38
Lead acetate 8.0 per cent during 60 days	4	♂	161 ± 10.6	1988 ± 23.6	589 ± 60.0	29
Lead acetate 12.0 per cent during 90 days	2	♀	192 ± 29.0	2628 ± 226.5	693 ± 72.5	81
Uninephrectomized				***		
Lead acetate 4.5 per cent during 20 days	5	♂	177 ± 15.4	1656 ± 133.4	462 ± 20.8	

* Standard error of the mean $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

** Body surface calculated from Meeh's formula: $S = 11.35\ W^{0.66}$

*** Weight of the only remaining kidney

TABLE II

Action of simultaneous administration of renotrophic agents and 4.5 per cent solution of lead acetate on kidney weight

SERIES	Nº rats	Sex	Body weight g	Kidney weight absolute mg	Kidney weight per 100 cm2 body surface mg	Percentage increase
Protein rich diet and lead acetate, 4 months	8	♀	160 ± 6.1	3239 ±186.0	964 ± 42.0	91
Protein rich diet, 4 months	4	♀	202 ± 11.1	1980 ±199.0	505 ± 15.5	
Unilateral nephrectomy, protein rich diet and lead acetate, 3 months	6	♀	171 ± 8.8	1816*± 75.5	520 ± 19.3	31
Unilateral nephrectomy and protein rich diet, 3 months	4	♀	191 ± 8.6	1497*± 86.4	398 ± 21.7	41
Thyroid powder and lead acetate, 3 months	5	♀	165 ± 4.8	2873 ±189.5	843 ± 60.0	
Thyroid powder, 3 months	6	♀	173 ± 6.2	2108 ± 66.0	597 ± 15.7	
Testosterone propionate, 2.5 mgs. per day and lead acetate, 29 days	6	♂	197 ± 5.5	2917 ± 53.0	760 ± 16.2	30**
Lead acetate, 29 days	5	♂	209 ± 8.9	2726 ±131.0	683 ± 27.3	49***
Testosterone propionate, 2.5 mgs per day, 29 days ..	4	♂	244 ± 10.5	2583 ±107.6	583 ± 9.0	28***
Unilateral nephrectomy, testosterone propionate 1 mg per day and lead acetate, 20 days	5	♂	178 ± 7.7	1767*±245.0	490 ± 17.0	
Unilateral nephrectomy and lead acetate, 20 days	5	♂	177 ± 15.4	1656*±133.4	462 ± 20.8	

* Weight of single remaining kidney

** Compared with rats receiving only testosterone propionate

*** Compared with normal controls (Table 1).

TABLE III

Inulin clearance (C_{in}) and diodrast tubular mass ($T_{m}D$) of rats poisoned with lead acetate (4.5 per cent)

SERIES	Nº rats	Sex	Body weight g	Kidney weight absolute mg	Kidney weight per 100 cm2 body surface mg	C /100 cm2 in			T /100 cm2 mD		
						Observed ml/min	Calculated** ml/min	Difference %	Observed ml/min	Calculated** ml/min	Difference %
Standard diet. Lead acetate during 12 months	2	♀	201 ± 8.5	2682 ± 212.5	690 ± 73.5	0.312	0.234	+ 44	0.121	0.100	+ 21
Unilateral nephrectomy. Lead acetate and protein rich diet during 3 months .	6	♂	198 ± 12.3	2267* ± 173.0	585* ± 25.0	0.294	0.333	— 4.6	0.141	0.109	+ 31.7
	2	♀	181 ± 29.5	1777* ± 152.0	490* ± 11.0	0.326	0.305	+ 7.3	0.122	0.104	+ 16.5
Unilateral nephrectomy and protein rich diet during 3 months (controls)	4	♀	195 ± 11.3	1497* ± 86.4	393* ± 25.1	0.344	0.424	— 17.7	0.098	0.125	— 20.5

* Right kidney weight

** Calculated from formula of Braun-Menéndez and Chiodi (1946) for normal rats.

compared with those described in the animals which only received the lead salt were less intense and less constant: in 4 of the 8 animals examined the kidneys were almost undamaged.

TABLE IV

Effect of dimercaptopropanol (BAL) on the kidney weight of rats poisoned with 4.5 per cent solution of lead acetate

SERIES	Nº rats	Sex	Body weight	Kidney weight absolute	Kidney weight per 100 cm2 body surface
			g	mg	mg
Lead acetate, 10 days	5	♂	116 ± 5.1	1894 ± 162.0	695 ± 44.0
Lead acetate and BAL, 10 days	5	♂	145 ± 4.2	1813 ± 129.0	577 ± 35.0
BAL, 10 days	3	♂	118 ± 13.5	1562 ± 184.0	570 ± 39.1
Lead acetate, 9 days	10	♀	114 ± 4.4	1471 ± 61.6	552 ± 18.4
Lead acetate and BAL, 9 days	9	♀	122 ± 3.5	1327 ± 40.9	474 ± 13.4
BAL, 9 days	6	♀	128 ± 3.5	1276 ± 39.6	441 ± 11.0
Normal controls	10	♀	128 ± 5.1	1138 ± 42.6	395 ± 10.8

Action of lead on the inulin clearance and diodrast tubular mass.

Determinations of inulin clearance and diodrast Tm were made in 6 uninephrectomized rats fed a protein rich diet and lead poisoned during 3 months and in 2 intact rats fed the standard diet and lead poisoned during 12 months. In all these animals values of diodrast Tm were from 16.5 to 31.7 per cent greater than the values obtained by Braun Menéndez and Chiodi (6) in normal animals. Inulin clearance was definitely increased only in the 2 rats which conserved both kidneys.

It is worth while pointing out that in the 4 control rats, uninephrectomized and fed a protein rich diet, the values of diodrast Tm and inulin clearance were lower than normal and similar to those obtained by Braun-Menéndez and Chiodi (7) in uninephrectomized rats fed a standard diet.

Action of BAL on the weight and histological structure of the kidney in rats poisoned with lead acetate.

Weight. — When a solution of dimercaptopropanol (BAL) was injected intraperitoneally at a daily dose of 66 mg/kg, simultaneously with the administration of 4.5 per cent solution of lead acetate by gastric tube, the kidney weight increase at the end of 10 days was very moderate or of no account. BAL alone produced a slight increase in kidney weight (table 4).

Histologic study. — Microscopic examination of the kidneys of 5 of the animals to which lead acetate and BAL were simultaneously administered

confirmed the results obtained by the study of kidney weight, lesions typical of lead poisoning were scarcely perceptible or absent. The administration of BAL alone produced generalized congestion with scanty albuminous exudate in the corpuscles in the 4 animals examined.

Action of lead on the blood pressure.

The blood pressure of the rats poisoned with lead in whichever the dose employed was always within the normal range with a tendency to lower values the longer the intoxication.

Discussion

Our results show that the ingestion of lead produces in the white rat apart from the classic focal destructive lesions in the renal tubules, an early kidney weight increase which persists on prolongation of lead administration up to a year.

This kidney size increase has all the characteristicis of a real hypertrophy, as shown by the increase of diodrast Tm and the hypertrophy, occasionally gigantic, of the tubular cells observed microscopically. These results confirm the findings of Calvery *et al,* of Finner and Calvery and of Chiodi and Sammartino. The hypertrophy we found was much greater than that described by Calvery and his associates. These authors found also an increasse in dry weight of the kidney which shows that there is real hypertrophy of the parenchima, an increase in mass, and not a simple imbibition of the tissues.

Renal hypertrophy seems to be more constant and marked with moderate (3 and 4.5 p. cent) than with the greater doses. Perhaps the cause lies in a lesser noxious action on the general health of the rats with the former.

The absence of well defined vascular and glomerular lesions is noteworthy, as well as the absence of renal sclerosis even in those animals which received the solution of lead acetate daily during one year. This fact, already pointed out by Calvery *et al.* and other authors, shows that in the rat lead is not the classical vascular poison and explains why hypertension was absent in our intoxicated rats.

Administration of a protein rich diet together with the lead salt reinforced markedly the renotrophic and renotoxic effect of the latter.

The association of thyroid powder or testosterone propionate with lead caused only a summation of effects, without potentiation of the renotrophic action of lead. On the contrary testosterone propionate had certain inhibitory effect on the forementioned renotrophic action as well as on the renotoxic effects as shown by the less marked and less constant histologic lesions.

In intoxications of short duration BAL prevented the kidney weight increase action of lead and diminished greatly the intensity of the microscopic lesions.

The nuclear and celular hypertrophy caused by lead cannot be a simple compensatory hyperthophy, secondary to the destruction of great number of tubular cells, because it was only rarely present in uranium

acetate poisoning in spite of an equal or greater intensity of the destructive lesions.

We believe the celular and nuclear hypertrophy caused by lead is due to a direct action of the metal, similar to that observed by Chiodi and Cardeza (10) in the liver of lead poisoned rats fed a fat rich diet. But proteins not only do not protect the kidneys as they did the liver, but furthermore they potentiate the action of the toxic agent.

CONCLUSIONS

Lead acetate per os produced in rats renal hypertrophy at the expense of tubular cells.

The renotrophic action of lead is believed to be specific, hypertrophy not being secondary to the destructive lesions caused by lead.

A protein rich diet potentiates the renotrophic action of lead.

Dimercaptopropanol (BAL) and in lesser grade testosterone propionate inhibit the renotrophic and renotoxic action of lead.

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ADMINISTRACION DE DESOXICORTICOSTERONA Y SENSIBILIDAD A LA HIPERTENSINA Y ADRENALINA EN RATAS

Por V. SILVA y H. CROXATTO

(Laboratorio de Fisiología, Universidad Católica, Santiago, Chile)

DESPUÉS de los trabajos de Selye (1946), se sabe que la administración de acetato de desoxicorticosterona (DC), tanto a pollos como a ratas, produce alteraciones nefroescleróticas, lesiones vasculares típicas e hipertensión, con lo cual la corteza suprarrenal ha debido ser considerada como un factor importante en el desarrollo de la enfermedad hipertensiva. Los hechos acumulados obligan a considerar una posible relación de la corteza suprarrenal con el mecanismo humoral de Goldblatt-Houssay.

Tratando de explicar el proceso por el cual la corteza suprerrenal podría favorecer el desarrollo de la enfermedad hipertensiva, hemos supuesto que las hormonas corticoadrenales, al menos la DC, podrían actuar en uno o varios de los siguientes mecanismos: influir a) en la liberación de renina por el riñón, b) en el rendimiento de la reacción renina-hipertensinógeno, c) en la inactivación de la hipertensina, d) en la sensibilización de los vasos al agente presor.

Las posibilidades b) y c) fueron estudiadas por Croxatto y Croxatto (1949) y los resultados de agregar "*in vitro*" DC a una mezcla de renina-hipertensinógeno y de hipertensina-hipertensinasa, han sido hasta ahora negativos. La investigación del punto a) no se ha intentado todavía en vista de que en la hipertensión esencial y en la experimental no existiría, al menos en los estados crónicos, un aumento de la renina circulante (Taquini y Fasciolo, 1947).

En el presente trabajo se ha tratado de investigar si la DC administrada a ratas por vía intravenosa es capaz de determinar un estado de mayor sensibilidad a la hipertensina. En vista del papel que juega la adrenalina en la regulación vasomotora, se estimó de interés estudiar en estos mismos animales la respuesta presora a la adrenalina. Además, considerando que los efectos descritos de la DC son más fáciles de determinar en animales uninefrectomizados y que reciben ClNa en abundancia, se incluyó en la investigación un cierto número de animales en los cuales se practicó inmediatamente antes del experimento la ligadura de ambos pedículos renales y se asoció a la administración de DC inyecciones de ClNa endovenosas.

Parte experimental

Se eligió ratas de peso comprendido entre 250 y 350 g, de ambos sexos, en las cuales, después de practicar la anestesia con Dial (10 mg por 100 g de peso), se controló en forma continua la presión arterial desde la arteria carótida. La observación de la presión arterial se hizo durante períodos largos, de 4 hasta 18 horas, sin interrupción. Después de poner en descubierto la vena femoral y seccionar el nervio crural a través de una pequeña incisión, se procedió en un segundo tiempo a seccionar la piel en la región del cuello para aislar la tráquea y la arteria carótida. Se practicó la termocauterización en todo punto donde se advirtió pérdidas de sangre. Se introdujo en la carótida primitiva una pequeña cánula constituída por un tubo de vidrio con una delgada punta en bisel y que poseía un tubito lateral cerrado con un manguito de goma. Por el otro extremo la cánula comunicaba con el sistema registrador de la presión, lleno con una solución de Tyrode que se renovaba cada vez. La presión se registraba con un manómetro de Hürtle, provisto de una membrana delgada, de pequeña superficie, sobre la cual descansaba una palanca que daba una ampliación de 10 veces. En cada experimento se calibraba el aparato por medio de un manómetro de mercurio. Un momento muy importante era el inyectar por la vena femoral, inmediatamente antes de introducir la cánula arterial, 0.3 ml de una solución de heparina (Abbott) que contenía 5 mg por ml. Además, la cánula debe llenarse previamente con esta misma solución de heparina. La tubuladura lateral de la cánula permitía introducir, por lo menos cada 4 horas, 0.4 a 0.5 ml de la solución anticoagulante.

La rata se reveló como un animal muy adecuado para observaciones de larga duración, siempre que se eviten las hemorragias y se controle la temperatura del animal. La presión arterial basal durante el curso de varias horas no experimentó variaciones bruscas o exageradas, pero en la mayoría de los casos se observó una disminución franca y paulatina. Para comparar entre sí los efectos de la administración de DC y los líquidos empleados para disolverla, se adoptó el criterio de medir:

a) la presión basal antes, durante y después de cada inyección de DC o sus disolventes;

b) el promedio del aumento tensional de dos o tres inyecciones sucesivas de hipertensina y de dos o tres inyecciones de adrenalina, antes y posteriormente a la inyección de DC y sus solventes;

c) los valores absolutos alcanzados por la presión con la introducción de cada substancia presora. Estos datos aparecen considerados en los gráficos y cálculos estadísticos.

Se estimó indispensable observar a los animales durante el mayor número de minutos posible, en vista de que los efectos de la administración de DC podrían requerir un tiempo largo para manifestarse.

La sensibilidad del animal a los agentes presores se estudió inyectando por vía endovenosa (femoral), dosis adecuadas de hipertensina y de

adrenalina que se administraron en series de tres inyecciones de una misma dosis de hipertensina, seguidas de otras tantas inyecciones de adrenalina. Estas series fueron repartidas antes, inmediatamente después y a intervalos de 30, 120, 180, 300, 500 minutos y más, después de haber inyectado la DC o los vehículos de la hormona cortical que se emplearon para disolverla e inyectarla.

Como estudio previo en un grupo de animales de control (serie A) se analizó en condiciones similares el efecto de la inyección de hipertensina y adrenalina, sin introducir ni la DC ni los respectivos solventes, con el objeto de establecer la posible existencia de variaciones espontáneas o imprevistas de la sensibilidad del animal al agente presor.

Material empleado

Hipertensina. — Se utilizó una solución stock que contenía 30 U por ml. Esta solución, diluída 10 veces en solución Tyrode, era inyectada en el animal por vía endovenosa generalmente en la proporción de 0.1 ml. En algunos casos la dilución se aumentó o disminuyó de acuerdo con la sensibilidad de la rata.

Adrenalina. — Se empleó solución de clorhidrato de adrenalina al 0.1 %, la que se diluía hasta 0.005 mg por ml.

Acetato de Desoxicorticosterona cristalizada Schering. — Esta fué disuelta en 3 diferentes solventes:

1) Propilén glicol "Carbon and Carbide", al cual se incorporó la DC disuelta en un pequeño volumen de éter que a continuación era eliminado por calentamiento. Se empleó dos concentraciones de DC: una correspondía a 10 mg por ml y otra a 0.4 mg por ml.

2) Tween 80 * (Sorbitan mono-oleato-poli-oxi alquileno). La DC se introdujo en el Tween disuelta en éter, que se eliminaba por calentamiento en baño de María a temperatura de ebullición, diluyéndose posteriormente (1:10) en agua destilada.

3) Alcohol absoluto. La solución alcohólica se preparó incorporando directamente la DC al alcohol en dosis de 0.25 mg por ml y diluyendo posteriormente 1:4 en agua destilada.

Metódica seguida

Como estos solventes se revelaron también capaces de despertar reacciones farmacológicas importantes, fué indispensable hacer un estudio especialmente orientado a determinar la influencia del solvente mismo sobre la presión arterial y sensibilidad de los animales al agente presor. Para esto se optó por realizar experimentos paralelos en condiciones semejantes sobre dos ratas en las cuales se hacía un registro separado de la presión arterial. Una de ellas servía de control para estudiar el efecto del solvente y la otra para el efecto del solvente más DC.

Aparte de la serie A, proyectada para controlar la sensibilidad de los animales a la hipertensina y adrenalina, se realizaron los siguientes experimentos que comprendieron las series:

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* Fabricado por la Atlas Powder Co., Delaware, U.S.A.

B) los animales inyectados con DC disuelta en propilenglicol;
C) los animales inyectados con propilenglicol;
D) los animales inyectados con DC disuelta en Tween;
E) los animales inyectados con Tween;
F) los animales inyectados con DC disuelta en alcohol etílico;
G) los animales inyectados con alcohol etílico.

En vista del hecho descrito que la administración de NaCl favorece el desarrollo de la hipertensión provocada por la DC, en la mayor parte de los animales de estas diversas series se practicó inyecciones intravenosas de NaCl al 10 %, de 1 a 3 ml en varias dosis y lentamente, antes o después de la administración de DC o de los diversos solventes.

Resultados

Serie A. — Respuesta presora a la hipertensina y adrenalina en ratas que no fueron inyectadas con DC ni con disolventes.

Se inyectaron 11 animales que se conservaron en buenas condiciones por lo menos durante 500 minutos.

Para evitar la deshidratación se inyectó NaCl al 9 ‰ por vía subcutánea en dos dosis de 5 ml cada una.

Las inyecciones de adrenalina e hipertensina practicadas en la vena femoral se iniciaron después que la presión arterial se estabilizó. Los efectos presores de esos principios varían de un animal a otro y al comienzo son más intensos cuando la presión basal es relativamente baja. En un mismo animal los efectos hipertensores fueron relativamente constantes, pero se observó una declinación paulatina de la respuesta en el curso del experimento, exceptuando 3 animales que después de los 120 minutos reaccionaron más intensamente a la adrenalina que al comienzo. Todos los animales dieron una respuesta progresivamente decreciente a la hipertensina.

En todo caso el valor absoluto de la presión alcanzado por las primeras inyecciones de estos dos principios vasoconstrictores fué siempre superior al de las últimas inyecciones. Examinando el gráfico 1, se puede apreciar en conjunto los valores promedios de las presiones basales y los cambios inducidos por la adrenalina e hipertensina.

Como puede apreciarse (gráfico 1), la presión basal del comienzo (9.8 ± 2.17 cm Hg), tiende a descender regularmente para llegar al final del experimento a 6.5 ± 1.66 cm Hg. Asimismo, los niveles de presión alcanzados por las inyecciones de hipertensina y adrenalina, que en un comienzo son de 12.6 ± 2.33 y 12.3 ± 2.31, respectivamente, declinan hacia el final a 8.7 ± 2.52 cm Hg y 10.3 ± 3.18 cm Hg.

Serie B. — Efectos de la hipertensina y adrenalina en animales inyectados con DC disuelta en propilenglicol.

Después de practicar series de inyecciones de dosis uniformes de adrenalina e hipertensina para conocer la sensibilidad de los animales a estos agentes presores, se procedió en 7 ratas a inyectar DC disuelta previamente en propilenglicol. Posteriormente, a intervalos de 25 y 150 minu-

tos, se repitieron las series de inyecciones de adrenalina e hipertensina en las mismas condiciones que al comienzo. En una rata las inyecciones se prolongaron hasta 420 minutos después de inyectada la DC. La solución de DC, 10 mg por ml, se inyectó en dosis de 0.2 ml con la mayor lentitud posible. La solución 0.4 mg por ml, previa dilución con agua destilada (1:4) al 25 %, se introdujo también lentamente en tres porciones. Estos últimos animales recibieron un total de 0.3 mg. Como puede observarse del análisis del gráfico 2, la inyección de DC en propilenglicol determina un

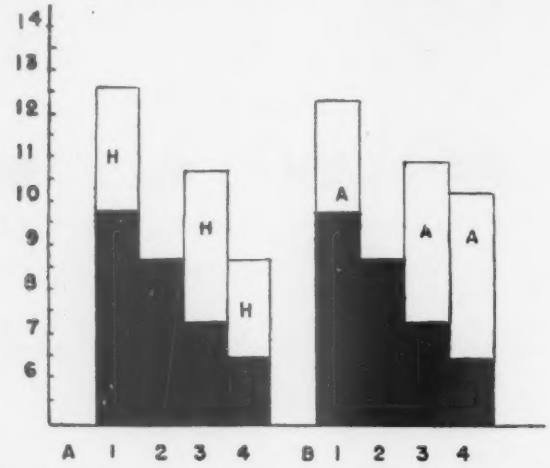

FIG. 1. — Valores promedios de la presión arterial de 11 ratas a lo largo de 500 minutos de observación. La ordenada indica la p.a. en cm de mercurio. La parte en negro del diagrama corresponde a la presión basal, y sobre ella, en claro, la respuesta a la hipertensina (H) y a la adrenalina (A). Las cifras del pie indican el tiempo transcurrido. 1: Momento inicial; 2: 60 minutos; 3: 210 minutos; 4: 500 minutos.

ascenso progresivo y duradero de la presión basal que tiende tardíamente a volver al nivel anterior. Si durante la primera hora que sigue a la inyección de DC, se inyecta adrenalina o hipertensina, se observa que éstas determinan un efecto presor más intenso que antes de la inyección de DC. Esta mayor sensibilidad a los agentes presores desaparece paulatinamente. Una segunda inyección de DC en propilenglicol reproduce, aunque en forma más atenuada, los efectos de la primera inyección. En 5 animales, antes o después de la DC, se inyectó 1 ml de NaCl al 10 %, lentamente, a razón de 0.2 ml por minuto, notándose un reforzamiento del efecto presor producido por la inyección de DC.

No obstante el aumento de la presión basal logrado por un período superior a 60 minutos en varios animales con la inyección de DC en propilenglicol, se pudo registrar que la presión basal descendió gradualmente en la última parte del experimento de una manera similar a la observada en las ratas de la serie preliminar (A).

Comparando la presión basal inicial y las observadas en los períodos intermedios y finales de estos animales con los de la serie anterior, no se encuentran diferencias significativas exceptuando la presión basal máxima alcanzada y la observada a los 25 minutos de inyectada la DC ($\Sigma = 2.43$). Los efectos presores de la hipertensina durante los primeros 30 a 60 minutos que siguen a las inyecciones de DC son más intensos que en ningún otro momento, si se consideran los valores absolutos de la pre-

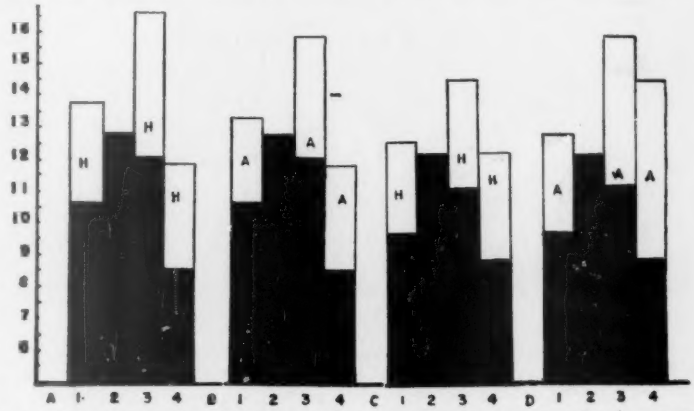

FIG. 2. — Valores promedios de la p.a. de 7 ratas inyectadas con DC. en propilenglicol y de 7 ratas inyectadas con propilenglicol solo.
1. Presiones iniciales.
2. Presiones basales máximas alcanzadas después de la inyección de DC o su solvente.
3. Id. a los 25 minutos después de la presión registrada en 2.
4. Id. después de 150 minutos.

Las demás abreviaturas tienen el mismo significado que en la figura 1.

sión, pero estadísticamente no se encuentra diferencia con los resultados registrados en condiciones semejantes en ratas inyectadas con propilenglicol solo.

Serie C. — Efecto de la inyección de propilenglicol.

En un grupo paralelo a la serie anterior de 7 animales se ensayó el efecto de la inyección de propilenglicol sin DC, inyectado puro, y diluído al 25 %, siguiendo el mismo orden de la serie B. El gráfico 2 permite confirmar el efecto descrito de esta substancia sobre la presión (Jarpa y col., 1948).

La comparación en el gráfico 2 de los promedios de las presiones en A. B, C, D y cálculos estadísticos revelan que no existen diferencias entre los resultados de esta serie (C) y la anterior (B) en lo que se refiere a la sensibilidad a la hipertensina y presión basal. En efecto, en estos animales la sensibilidad a los agentes vaso constrictores evoluciona en forma muy cercana a la de la serie anterior, por lo que puede deducirse que las modificaciones registradas no son dependientes de la introducción de DC sino del propio solvente.

También en este grupo de ratas, como en el precedente, la presión evolucionó hacia el final como en el de la serie preliminar; así, la comparación de las presiones basales a los 150 minutos de la inyección de propilenglicol o propilenglicol con DC, de la serie B y C, con las presiones que corresponden a los 210 minutos de la serie preliminar A, no ofrece diferencia significativa.

Esto demostraría que la DC o el solvente empleado no modifican las condiciones circulatorias de la rata dentro del lapso que duraron las observaciones, exceptuando el efecto inmediato (30 a 60 min.).

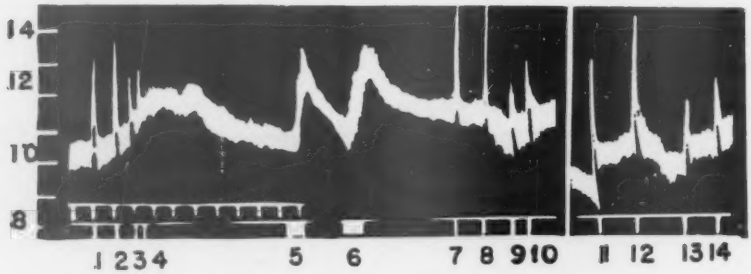

FIG. 3. — Presión arterial de una rata anestesiada con Dial. Respuesta a la Hipertensina y Adrenalina antes y después de inyectar DC disuelta en Tween. La curva superior del gráfico corresponde a la p.a. La línea intermedia corresponde al tiempo; cada espacio indica 5 minutos. La línea inferior marca los momentos en que se practican las inyecciones.

En: 1-2) Hipertesina 0.3 U. 3-4) Adrenalina 0.5 microgramos. 5) 1 mg de DC en 0.5 ml Tween 10 %. 6) 1 ml NaCl 10 %. 7-8) Hipertensina 0.3 U. 9-10) Adrenalina 0.5 microgramos. Entre 10 y 11 transcurren 60 minutos. 11-12) Hipertensina 0.3 U. 13-14) Adrenalina 0.5 microgramos.

Serie D. — Efecto de la inyección de DC disuelto en "Tween 80".

Esta serie comprendió 19 animales. La solución que se inyectaba al animal contenía 1 mg de DC por ml. Se inyectaba 0.25 a 0.5 ml, o sea de 0.250 mg a 0.5 mg administrados en pequeñas porciones.

El gráfico 3 revela que la inyección de Tween con DC modifica la presión basal y la sensibilidad del animal a los principios hipertensores de una manera semejante a la descrita en la parte B y C. La comparación de esta serie D con la serie preliminar da una sorprendente similitud con lo ya encontrado en el estudio comparativo de los grupos anteriores ($\Sigma = 2.08$). Resultan del mismo orden los aumentos máximos de la presión basal en esta serie D con los observados en la serie E donde se estudia el efecto del Tween sin DC.

En 12 animales de este grupo D se practicó previamente la ligadura de los pedículos renales y en 10 de ellos se administró NaCl al 10 % en dosis de 1 ml, inyectado en diversas porciones algunos minutos antes o después de introducir la DC en Tween. No se observó diferencia en cuanto a este efecto presor inmediato con lo encontrado en los animales con pedículos renales no ligados. Sin embargo, la presión basal de los animales nefrectomizados tiende a elevarse gradualmente, para llegar

en los períodos terminales a valores superiores a los registrados al comienzo del experimento.

Serie E. — Efecto del Tween sin DC.

Comprendió un grupo de 10 animales de los cuales a 4 se ligó los pedículos renales y a 4 se inyectó NaCl. El estudio comparativo de los

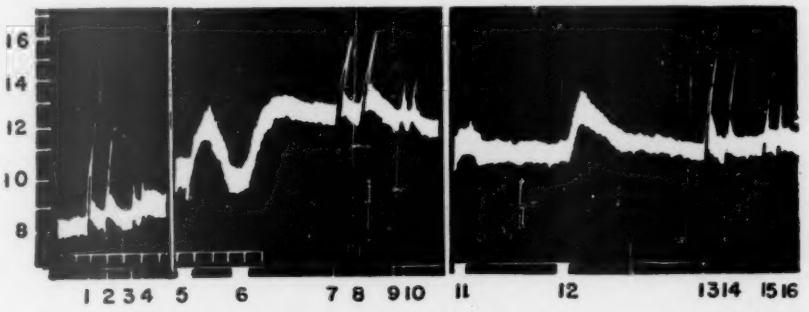

FIG. 4. — Presión arterial de una rata anestesiada con Dial. Respuesta a la Hipertensina y Adrenalina antes y después de inyectar Tween solo. Las indicaciones son idénticas a las de la figura 3.

En: 1 - 2) Hipertensina 0.3 U.
3 - 4) Adrenalina 0.5 microgramos. Entre 4 y 5 transcurren 30 minutos.
5) 0.25 ml Tween 10 %.
6) 2 ml NaCl 10 %.
7 - 8) Hipertensina 0.3 U.
9 - 10) Adrenalina 0.5 microgramos. Entre 10 y 11 transcurren 60 minutos.
11) 0.25 ml Tween 10 %.
12) 1 ml NaCl 10 %.
13 - 14) Hipertensina 0.3 U.
15 - 16) Adrenalina 0.5 microgramos.

cambios de las presiones basales y de la sensibilidad a la hipertensina y adrenalina no dió diferencia significativa con la serie de Tween con DC. (Gráfico 4).

Serie F. — Efecto de la inyección de DC disuelta en alcohol.

Se empleó tres animales que fueron inyectados con una solución de DC en alcohol en diversas dosis de 200 y 400 microgramos por animal, que se inyectaban lentamente por vía intravenosa en pequeñas dosis con un volumen total de 1 a 4 ml. Las reacciones presoras a la hipertensina, después de 30 minutos de transcurrida la inyección de la solución alcohólica de DC, aumentaron en dos animales. En el restante se observó un ligero descenso. Después de los 90 minutos de inyectada la DC la respuesta a los agentes presores se mantiene algo superior en un caso.

El hecho más típico de la inyección de DC en alcohol es el aumento de la presión basal, y que dura cierto tiempo.

Este aumento de la presión basal no se repite si se practica la inyección de una segunda dosis de DC y tampoco se obtiene una respuesta

más intensa a la hipertensina y adrenalina. El aumento de la presión basal es fenómeno que no puede atribuirse a la DC, porque, como se verá en la serie siguiente, también se produce con la misma intensidad por la inyección de alcohol sin DC.

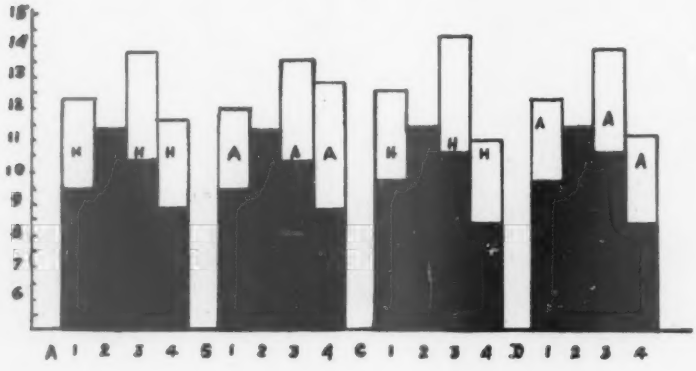

FIG. 5. — Promedio de las presiones arteriales en 29 ratas inyectadas con DC de los grupos B, D y F y de 20 ratas inyectadas con los solventes sin DC, de los grupos C, E, G.

En: 1) Presiones iniciales.
2) Presiones máximas alcanzadas después de la DC o sus solventes.
3) Presiones a los 30 minutos después de 2).
4) Presiones a los 90 a 150 minutos después de 3).
Grupo inyectado con DC.
A) respuesta presora a la hipertensina.
B) respuesta presora a la adrenalina.
Grupo inyectado con el solvente.
C) respuesta presora a la hipertensina.
D) respuesta presora a la adrenalina.
Los cálculos estadísticos no revelan diferencias significativas entre ambos grupos, tanto en lo que se refiere a las presiones basales como al comportamiento de la presión arterial después de inyectar hipertensina o adrenalina.

Serie G. — Efecto de la inyección de alcohol.

En 3 ratas, que se estudiaron paralelamente con las de la serie A, se obtuvo con la simple inyección de alcohol absoluto diluído al 25 % y administrado en forma semejante a la solución de DC, un resultado prácticamente igual. La mayor respuesta a la hipertensina y adrenalina se obtuvo dentro de los 30 minutos de inyectado el alcohol a los animales. Es probable que la DC en la serie F no tuviera influencia apreciable en el comportamiento de la presión dentro del tiempo que duró la observación.

Para apreciar comparativamente, en una visión de conjunto, los efec-

tos de los solventes empleados, con los obtenidos cuando se agrega DC, se agruparon en el gráfico 5 los resultados obtenidos en los 29 animales inyectados con DC en los tres diferentes solventes, con los promedios de los 20 animales inyectados exclusivamente con los solventes: propilenglicol, Tween, alcohol etílico. Se puede apreciar que tanto la presión basal, como los efectos de la hipertensina y adrenalina, antes y después de las inyecciones de cada solución de DC o de los respectivos solventes puros se modifican de manera semejante en ambos grupos.

Discusión

El hecho que vale la pena destacar en primer término, es que la DC no indujo por sí sola ningún aumento de sensibilidad del animal a los agentes vasoconstrictores. Aun cuando la observación de los animales no se prolongó en la mayoría más allá de 150 minutos de la inyección de DC o sus solventes, hubo algunos que fueron estudiados hasta 540 minutos después de la primera inyección de DC. Podría suponerse que la DC administratada por vía intravenosa disuelta en los líquidos orgánicos elegidos sea rápidamente excluída de la circulación en vista de la escasa solubilidad de la hormona en los humores, pero eso estaría en contradicción con algunas observaciones realizadas por Croxatto y Silva (1949), en sapos que revelaron que los efectos anestésicos de la DC disuelta en Tween e inyectada por vía intracardíaca son más rápidos y más intensos que los obtenidos con las inyecciones oleosas por vía subcutánea.

Goldman y Schroeder (1948), inyectando en el hombre DC disuelta en propilenglicol por vía intravenosa, han pretendido demostrar que la DC tiene efecto presor inmediato en enfermos hipertensos. Sin embargo, estos resultados son discutibles desde el momento que el análisis estadístico de los mismos no permite encontrar diferencia significativa entre los inyectados con propilenglicol solo y los que reciben la hormona en propilenglicol.

Es posible admitir que los resultados negativos obtenidos en nuestros experimentos, en lo que concierne al efecto de la DC sobre la reactividad de los vasos al agente presor, se expliquen porque esta acción requiera de un mecanismo indirecto de lento desarrollo, superior a 3 ó más horas después de inyectada y que la hipertensión que se desarrolla en los animales sometidos a las condiciones descritas por Selye se explica por la concurrencia de factores que no se es posible reunir en nuestros experimentos. En todo caso parece muy poco probable que la DC tenga alguna acción directa sobre los vasos que condicione una mayor sensibilidad de éstos a la adrenalina o hipertensina. La presencia de los riñones juega un papel de importancia en la presión basal, la que tiende a mantenerse o a subir ligeramente al final del experimento, especialmente si se asocia a la DC la administración de NaCl al 10 %.

Conclusiones

En 11 ratas anestesiadas, en las cuales se controló directamente la presión arterial en la carótida, se estudió la intensidad de la respuesta presora a inyecciones intravenosas de adrenalina e hipertensina, administradas a intervalos en el curso de 3 a 8 horas de observación. Se pudo

registrar que la presión basal de estos animales va declinando paulatinamente y que disminuye en forma gradual la reacción hipertensora producida por la hipertensina y la adrenalina.

En 29 ratas, en las cuales se administró DC en dosis de 0.3 a 2 mg., disuelta en propilenglicol (7), Tween 80 (19), alcohol (3), por vía intravenosa, pudo observarse que la presión basal sube y se mantiene más elevada durante un tiempo de 15 a 90 minutos para restablecerse al nivel anterior. Durante este período los animales reaccionan con mayor intensidad (diferencia significativa) a los agentes presores estudiados.

Estos efectos no pueden ser atribuídos a la DC porque reacciones semejantes y de la misma intensidad se obtuvieron con la inyección de los disolventes, propilenglicol, Tween y alcohol etílico sin DC.

La eliminación de ambos riñones y la administración de NaCl al 10 % hacen en general más apreciables los efectos sobre la presión basal de las soluciones de DC y de sus disolventes. Además, en estos animales así tratados la presión basal tiende a mantenerse sin declinar durante un mayor tiempo y aún a subir paulatinamente hacia los períodos finales.

SUMMARY

In 11 anaesthetized rats the pressor response to intravenous injections of adrenaline and hypertensin (administered at intervals during a period of 3 to 8 hours), was studied by means of a direct control of the blood pressure of the internal carotid artery. It was observed that the basal blood pressure of these animals slowly declined, also that there was a gradual decrease in the hypertensive reaction to hypertensin and adrenaline.

In 29 rats which had received doses of 0.3 to 2 mg of DC dissolved in proyleneglycol (7), Tween 80 (19), and alcohol (3) intravenously, it was observed that the basal blood pressure rose and remained high for 15 to 90 minutes before returning to its previous level. During this period the animals reacted with increased intensity to the above mentioned pressor agents.

This effect cannot be attributed to the DC because similar reactions were observed by the use of the solvents, propyleneglycol, Tween and ethyl alcohol without DC.

Elimination of both kidneys and the administration of a 10 % solution of NaCl increases the pressor effect of DC and its solvents. Moreover, in these animals the basal pressure tends to remain high over a longer period and even increases slowly towards the end.

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LIVER URIDINE PHOSPHORYLASE

By C. E. Cardini, A. C. Paladini, R. Caputto, and L. F. Leloir

(Instituto de Investigaciones Bioquímicas, Fundación Campomar, Julián Alvarez 1719, Buenos Aires, Argentina).

Some animal tissues contain enzymes which split off the base from nucleosides. Klein (1) studied their action on purine and pyrimidine nucleosides and called them nucleosidases. He observed that their activity disappears after dialysis and reappears in the presence of phosphate or arseniate.

The mechanism of action of one of these enzymes was elucidated by Kalckar (2). In the course of studies on the estimation of nucleosides (3) he rediscovered the necessity of phosphate and was able to prove that ribose-1-phosphate was formed in the reaction. The latter substance is very acid-labile, so that the phosphate estimations had to be carried out with the method of Lowry and López (4) which avoids the use of strong acid.

Kalckar found that with a rat liver enzyme and inosine (hypoxanthine riboside) or guanosine as substrates, the following reversible reactions took place:

$$\text{Hypoxanthine riboside} + \text{phosphate} \rightleftarrows \text{hypoxanthine} + \text{ribose-1-phosphate}$$

$$\text{Guanosine} + \text{phosphate} \rightleftarrows \text{guanine} + \text{ribose-1-phosphate}$$

Due to the similarity of the reaction with that catalyzed by polysaccharide phosphorylase the enzyme was named nucleoside phosphorylase. The ribose-1-phosphate formed appears to be the furanoside since it was found that synthetic ribopyranose-1-phosphate was not used in the reaction going from right to left, that is in the synthesis of nucleosides.

Kalckar was unable to detect any action of the enzyme on adenosine, xanthosine or pyrimidine ribosides. However, the experiments of Schlenk and Waldvogel (5) show that ribose-phosphate is formed also from adenosine.

Similar reactions have been found to occur with desoxyribose-nucleosides. Manson and Lampen (6) obtained an enzyme from thymus which catalyzed the formation of desoxyribose-phosphate from hypoxanthine desoxyriboside and inorganic phosphate. The ester formed was believed to be desoxyribose-5-phosphate. They also detected a similar enzyme in bone marrow and kidney which acts on desoxypyrimidine-nucleosides, specially thymidine (7). Similar results have been obtained by Wajzer (8).

The formation of desoxyribose-1-phosphate from guanine-desoxyriboside was detected by Friedkin, Kalckar and Hoff-Jorgensen (9). The reaction was found to be reversible and the nucleoside could be synthesized from hypoxanthine and desoxyribose-1-phosphate (2). The latter substance was found to be even more acid-labile than ribose-1-phosphate.

Crude extracts of liver and other tissues catalyze the formation of ribose-5-phosphate from the l-isomer. This reaction is similar to the transformation of the glucose-1-phosphate into the 6-phosphate which requires glucose-1,6-diphosphate as coenzyme ([10]). It is therefore likely that the coenzyme of phosphoribomutase is ribose-1,5-diphosphate ([11]). The existence of a similar enzyme for the desoxyribose-phosphate has been postulated by Manson and Lampen (6).

Schlenk and Waldvogel ([5]) have observed that when guanosine or adenosine is incubated with liver extracts and phosphate, the ribose disappears and is replaced by an acid stable phosphoric ester. They were able to prove ([12]) that about half the ribose-phosphate was transformed into hexose-6-phosphate. The same transformation occurred starting with ribose-5-phosphate but not with free ribose or ribose-3-phosphate.

A similar observation had been made by Dische ([13]) several years before. In human red blood cells the ribose of adenosine disappeared. He suggested that the pentose was transformed into triose-phosphate and a two carbon compound. Using a bacterial enzyme Racker ([14]) detected the formation of triose-phosphate from ribose-5-phosphate. With muscle aldolase he observed a condensation of triose-phosphate with glycollic aldehyde to a pentose-phosphate. However, the product was not ribose-phosphate nor was there any evidence of its transformation into this substance.

The synthesis of inosinic acid has been reported by Wajzer and Baron ([15, 16]) by incubation of inosine, ribose-3-phosphate and a liver enzyme. The inosinic acid was estimated by its activating action of polysaccharide phosphorylase.

Interest in the metabolism of uridine was aroused in this laboratory as a consequence of the isolation of uridine-diphosphate-glucose ([17]) which acts as a coenzyme in the galactose-1-phosphate → glucose-1-phosphate transformation. It was found that rat liver contains an enzyme which removes uracil from ribose, which is activated by phosphate and which leads to the formation of ribose-phosphate. The enzyme therefore catalyzes the phosphorolysis of uridine. In a note Paege and Schlenk ([18]) have recently reported the presence of a similar enzyme in bacteria.

Experimental

Preparation of the enzyme. — Rats were killed and the liver was immediately homogeneized in two volumes of water. After standing two or three hours in the ice-box, the homogenate was centrifuged at 3 000 r.p.m. The supernatant was then dialyzed against distilled water for 15 to 20 hours. Dialysis for a longer time did not inactivate the enzyme. On storage for several days at —5°C inactive protein precipitated and no appreciable loss of activity was observed. In some experiments the extract of acetone dried liver as described by Schlenk and Waldvogel ([5]) was used, and in others the extract prepared according to Kalckar ([3]) taking the fraction which precipitates between 0.4 and 0.6 saturated ammonium sulphate followed by dialysis.

TABLE I

Action of the liver extract on different substrates. Results in micromoles of ribose-5-phosphate

SUBSTRATE	Hours of incubation			
	1	2	3	5
Uridine	0.14	0.25	0.29	0.29
Uridine-3'-phosphate	0.12	0.17	0.21	0.24
Uridine-5'-phosphate	—	—	0.29	—
Cytosine	0	0	0	—
Cytosine-3'-phosphate	0	0	0	—

TABLE II

The action of phosphate and magnesium. Procedure as described in text. Substrate: uridine. Results in micromoles of ribose-5-phosphate

Hours of incubation	No phosphate No magnesium	No phosphate With magnesium	With phosphate With magnesium
1	0	0.02	0.15
2	0.03	0.02	0.16

TABLE III

The action of magnesium icn concentration. Procedure as described in text. Incubation during 2 hcurs at 37°C. Results in micromoles cf ribose-5-phosphate

Molar concentration of Mg	0	.0013	.0025	.005	.01	0.02	0.04
Uridine	.09	—	0.12	0.15	0.20	0.22	0.22
Uridine-3'-phospha-te	.04	0.07	0.10	0.11	0.15	0.18	—

TABLE IV

pH optimum. Results in micromoles of ribose-5-phosphate

SUBSTRATE	pH			
	6.6	7.0	7.4	8.0
Uridine	0.06	0.08	0.18	0.17
Uridylic acid.	0.11	0.12	0.13	0.11

Substrates. — Uridine, uridine-3'-phosphate, cytosine and cytosine-3'-phosphate were prepared according to Loring *et al* ([19]) or were commercial samples (Schwarz). Uridine-5'-phosphate was prepared from uridine-diphosphate-glucose ([17]).

Estimation of the enzyme. — The ribose of pyrimidin-ribosides gives no colour with the methods used for pentose estimation. Therefore, the liberation of ribose or ribose-phosphate can be easily detected.

The enzymatic system was made up as follows: 1 micromole of substrate, plus 0.1 ml of 0.06 M phosphate buffer of pH 7.4 plus 0.1 ml of 0.08 M magnesium chloride and 0.1 ml of enzyme solution. Total volume, 0.3 to 0.4 ml. Incubation was carried out at 37°C in the presence of toluene.

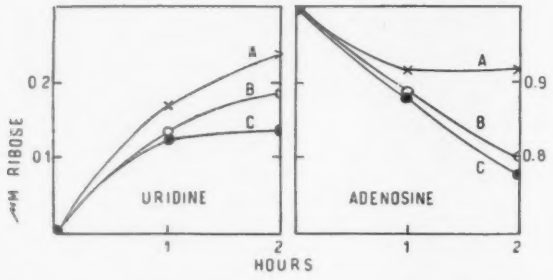

FIGURE 1. — The action of different enzyme preparations on uridine and adenosine.
x Liver extract dialyzed 20 hours.
• Fraction precipitating between 0.4 – 0.6 saturation with ammonium sulphate following the procedure of Kalckar (3).
o Extract of acetone dried liver according to Schlenk and Waldvogel (5).

The reaction was interrupted at the desired time by addition of one volume of 2 N HCl; after centrifugation an aliquot of the supernatant, usually half, was taken. Water was then added to make 3 ml and the ribose was estimated following the method described by Mejbaum ([20]), heating 30 minutes. In some cases the coloured compound was extracted with amyl alcohol. Ribose or xylose were used as standards and the colorimetric values were calculated as ribose-5-phosphate following the indications of Albaum and Umbreit ([21]).

RESULTS

Action on different substrates. — As shown in table I, the liberation of ribose could be detected with uridine or its phosphates, but not with cytosine. With the latter the addition of heated liver extracts was tested with negative results. The uridine-phosphates are probably transformed into uridine by phosphatase action.

Accurate results of the activity of uridine-phosphorylase were not obtained because the system contains enzymes which catalyze the disappearance of ribose-phosphate. This can be observed in fig. 1. When adenosine is the substrate the ribose moiety is directly estimated with Mej-

baum's procedure. By incubation with the enzyme mixture the values decrease due to the further transformation of the ribose-phosphate liberated by the phosphorylase. With uridine the ribose moiety is not estimated until it is transformed into ribose-phosphate. The accumulation of ribose-phosphate from uridine depends on the relative activity of two reactions: the uridine-phosphorylase which forms ribose-phosphate and the other enzymes which destroy it. The results using the enzyme preparations of Kalckar and Schlenk and Waldvogel are also shown in fig. 1.

Conditions for maximum activity. — Results appearing in table II show that practically no enzymatic activity was detected in the absence of either phosphate or magnesium ions. The effect of the latter was more

TABLE V

The formation of ribose esters. Procedure described in text. Amount doubled. Substrate: uridine. Ribose esters represent the part precipitated by the Somogyi deproteinizing procedure.

Hours of incubation	Free ribose μ moles	Ribose esters μ moles
1	0.04	0.30
2	0.04	0.50
3	0.06	0.53

TABLE VI

Liberation of uracil. Substrate: 2 micromoles of uridine. Uracil estimated by absorbency at 290 mμ in 0.01 NaOH after deproteinizing with trichloracetic acid.

Hours of incubation	Ribose esters formed	Uracil liberated
2	0.40	0.46
3	0.46	0.54

manifest after a thorough dialysis. The optimum concentration of magnesium is 0.02 to 0.04 M or higher (table III).

The pH optimum was about 7.4 both when the substrate was uridine or uridylic acid (table IV).

The formation of ribose ester. — It is known that carbohydrate phosphates are precipitated in the deproteinization procedure described by Somogyi ([22]). Use of this fact was made in order to find out whether the ribose liberated from uridine was free or esterified. The ribose left

in the supernatant after zinc sulphate barium hydroxide treatment was substracted from the amount found directly. This value was considered to represent esterified ribose.

As shown in table V, practically all the ribose liberated by the enzyme is in the bound form.

The liberation of uracil. — The absorption spectra of uracil and uridine published by Ploeser and Loring ([23]) show that in 0.01 N sodium hydroxide the molar absorbency index (molar extinction coefficient) at 290 mμ for uracil is 5 500 and practically zero for uridine. It is thus possible to estimate these two substances in a mixture. As shown in table VI, the amount of uracil liberated is slightly higher than the amount of ribose esters formed.

Discussion

The reactions catalyzed by the enzyme mixture can be formulated as follows:

I Uridine + phosphate $\rightleftarrows$ uracil + ribose-1-phosphate.
II Ribose-1-phosphate $\rightleftarrows$ ribose-5-phosphate.
III Ribose-5-phosphate $\rightleftarrows$ unidentified products.

That reaction I takes place is proved by the liberation of uracil, by the indispensability of phosphate for the reaction and by the formation of ribose ester. Reactions II and III also take place with the extract used because starting with adenosine there occurs a disappearance of ribose, the mechanism of which has been studied by other workers.

The necessity of magnesium ions has not been mentioned in the work of Kalckar on guanosine-phosphorylase. It is likely that magnesium is necessary for reaction II and not for the phosphorylase (reaction I). The activation observed would be due to the fact that reaction I is reversible and would not take place if the ribose-1-phosphate is not removed from the reaction mixture by the phosphoribomutase (reaction II).

The activity of nucleoside phosphorylase which was found by Kalckar to be limited to the guanine and hypoxanthine ribosides, appears to be more general. Thus activity on adenosine was found by Schlenk and coworkers and activity on uridine is described in this paper. It is likely that the discrepancies are due to the methods of estimation and on the position of the equilibrium in these reactions.

Another point which remains to be settled is whether there is only one phosphorylase which acts on several ribosides or whether each nucleoside needs a specific enzyme.

Summary

Rat liver extracts were found to contain an enzyme which in the presence of inorganic phosphate transforms uridine into uracil and ribose phosphate. The process was found to be accelerated by magnesium ions.

Uridylic acid was also transformed whereas cytosine and cytidylic acid were not.

RESUMEN

En el extracto de hígado de rata se demostró la presencia de una fosforilasa que actúa sobre la uridina y los ácidos uridílicos originando, en presencia de fosfato y magnesio, uracilo y ésteres de ribosa.

La citosina y el ácido citidílico no son atacados.

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(3) GOLDBERGER, E.: *Unipolar lead electrocardiography*. Philadelphia, Lea and Febiger, 1947.

(4) GÓMEZ, S. L., PÉREZ, J. M., LÓPEZ, N. A.: *Acta physiol. Lat.-Amer.*, 1950, *1*, 43.

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metro	m	litro	l	microgramo	µg
centímetro	cm	centímetro cúbico	cm^3	gamma	γ
milímetro	mm	mililitro	ml	por ciento	%
micrón	µ	kilogramo	kg	hora	h
milimicrón	mµ	gramo	g	minuto	m
Angström	A°	miligramo	mg	segundo	s
				milisegundo	ms

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